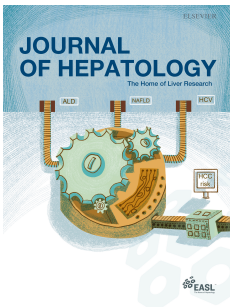
Journal Pre-proof



Prognosis of patients with hepatocellular carcinoma treated with immunotherapy – development and validation of the CRAFITY score

Bernhard Scheiner, Katharina Pomej, Martha M. Kirstein, Florian Hucke, Fabian Finkelmeier, Oliver Waidmann, Vera Himmelsbach, Kornelius Schulze, Johann von Felden, Thorben W. Fründt, Marc Stadler, Harald Heinzl, Kateryna Shmanko, Stephan Spahn, Pompilia Radu, Alexander R. Siebenhüner, Joachim C. Mertens, Nuh N. Rahbari, Fabian Kütting, Dirk-Thomas Waldschmidt, Matthias P. Ebert, Andreas Teufel, Sara De Dosso, David J. Pinato, Tiziana Pressiani, Tobias Meischl, Lorenz Balcar, Christian Müller, Mattias Mandorfer, Thomas Reiberger, Michael Trauner, Nicola Personeni, Lorenza Rimassa, Michael Bitzer, Jörg Trojan, Arndt Weinmann, Henning Wege, Jean-François Dufour, Markus Peck-Radosavljevic, Arndt Vogel, Matthias Pinter

PII: S0168-8278(21)02100-0

DOI: https://doi.org/10.1016/j.jhep.2021.09.035

Reference: JHEPAT 8459

To appear in: *Journal of Hepatology*

Received Date: 14 January 2021

Revised Date: 20 September 2021

Accepted Date: 26 September 2021

Please cite this article as: Scheiner B, Pomej K, Kirstein MM, Hucke F, Finkelmeier F, Waidmann O, Himmelsbach V, Schulze K, von Felden J, Fründt TW, Stadler M, Heinzl H, Shmanko K, Spahn S, Radu P, Siebenhüner AR, Mertens JC, Rahbari NN, Kütting F, Waldschmidt DT, Ebert MP, Teufel A, De Dosso S, Pinato DJ, Pressiani T, Meischl T, Balcar L, Müller C, Mandorfer M, Reiberger T, Trauner M, Personeni N, Rimassa L, Bitzer M, Trojan J, Weinmann A, Wege H, Dufour JF, Peck-Radosavljevic M, Vogel A, Pinter M, Prognosis of patients with hepatocellular carcinoma treated with immunotherapy – development and validation of the CRAFITY score, *Journal of Hepatology* (2021), doi: https://doi.org/10.1016/j.jhep.2021.09.035.

Prognosis of patients with HCC treated with immunotherapy – the CRAFITY score

A simple score based on C-reactive protein (CRP) and alpha-fetoprotein (AFP) identifies patients with favourable survival

CRAFITY (CRP & AFP in ImmunoTherapY) score

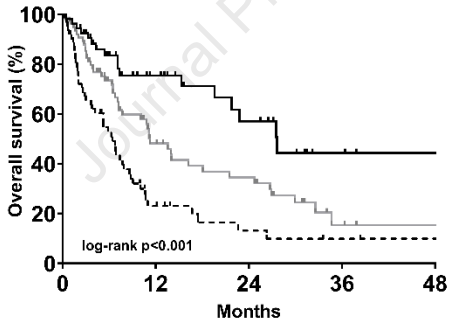
- **AFP ≥100 ng/mL**: 1 point
- **CRP ≥1 mg/dl**: 1 point

CRAFITY-low: 0 points
CRAFITY-intermediate: 1 point
CRAFITY-high: 2 points

European multicenter study:

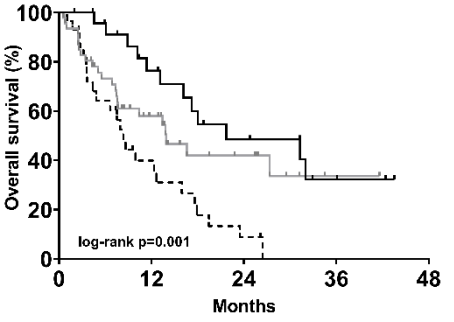
- **Training cohort**:
  6 centers – 190 patients
- **Validation cohort**:
  8 centers – 102 patients

Overall survival according to CRAFITY score (training cohort)



CRAFITY-low: median 27.6 months (95%CI, 19.5-35.8)
CRAFITY-intermediate: median 11.3 months (95%CI, 8.0-14.6)
CRAFITY-high: median 6.4 months (95%CI, 4.8-8.1)

Overall survival according to CRAFITY score (validation cohort)



CRAFITY-low: median 21.7 months (95%CI, 5.5-38.0)
CRAFITY-intermediate: median 13.9 months (95%CI, 7.4-20.4)
CRAFITY-high: median 8.4 months (95%CI, 6.6-10.1)

Scheiner B et al. J Hepatol 2021

MEDICAL UNIVERSITY OF VIENNA

Prognosis of patients with hepatocellular carcinoma treated with immunotherapy – development and validation of the CRAFITY score

Bernhard Scheiner[1,2], Katharina Pomej[1,2], Martha M Kirstein[3,4], Florian Hucke[5], Fabian Finkelmeier[6], Oliver Waidmann[6], Vera Himmelsbach[6], Kornelius Schulze[7], Johann von Felden[7], Thorben W. Fründt[7], Marc Stadler[2,8], Harald Heinzl[8], Kateryna Shmanko[9], Stephan Spahn[10], Pompilia Radu[11,12], Alexander R. Siebenhüner[13,14], Joachim C. Mertens[15], Nuh N. Rahbari[16], Fabian Kütting[17], Dirk-Thomas Waldschmidt[17], Matthias P. Ebert[18,19], Andreas Teufel[19,20], Sara De Dosso[21], David J. Pinato[22,23], Tiziana Pressiani[24] Tobias Meischl[1,2], Lorenz Balcar[1,2], Christian Müller[1,2], Mattias Mandorfer[1], Thomas Reiberger[1,25,26,27,28], Michael Trauner[1], Nicola Personeni[24,29], Lorenza Rimassa[24,29], Michael Bitzer[10], Jörg Trojan[6], Arndt Weinmann[9], Henning Wege[7,30], Jean-François Dufour[11,12], Markus Peck-Radosavljevic[5], Arndt Vogel[3], Matthias Pinter[1,2]

[1] Division of Gastroenterology and Hepatology, Department of Internal Medicine III, Medical University of Vienna, Vienna, Austria

[2] Liver Cancer (HCC) Study Group Vienna, Medical University of Vienna, Vienna, Austria

[3] Department of Gastroenterology, Hepatology and Endocrinology, Hannover Medical School, Hannover, Germany

[4] Department of Medicine I, University Medical Center Schleswig-Holstein, Lübeck, Germany

[5] Internal Medicine and Gastroenterology (IMuG), Hepatology, Endocrinology, Rheumatology and Nephrology including Centralized Emergency Department (ZAE), Klinikum Klagenfurt am Wörthersee, Klagenfurt, Austria

[6] Department of Gastroenterology, Hepatology and Endocrinology, University Hospital Frankfurt, Frankfurt/Main, Germany

[7] 1. Department of Internal Medicine, Gastroenterology & Hepatology, University Medical Center Hamburg-Eppendorf, Hamburg, Germany

[8] Center for Medical Statistics, Informatics and Intelligent Systems, Medical University of Vienna, Vienna, Austria

[9] Department of Internal Medicine I, University Medical Center of the Johannes Gutenberg University Mainz, Mainz, Germany.

[10] Department Internal Medicine I, Eberhard-Karls University, Tuebingen, Germany

[11] Hepatology-Department of Biomedical Research, University of Bern, Bern, Switzerland

[12] University Clinic for Visceral Surgery and Medicine, Inselspital, University of Bern, Bern, Switzerland

[13] Department of Medical Oncology and Hematology, University Hospital Zurich and University Zurich, Zurich, Switzerland

[14] Department of Medical Oncology and Hematology, Cantonal Hospital Schaffhausen, Schaffhausen, Switzerland

[15] Department of Hepatology and Gastroenterology, University Hospital Zurich and University Zurich, Zurich, Switzerland

[16] Department of Surgery at University Hospital Mannheim, Medical Faculty Mannheim, Heidelberg University, Mannheim, Germany

[17] Department of Gastroenterology and Hepatology, University of Cologne, Cologne, Germany

[18] Department of Internal Medicine II, University Medical Center Mannheim, Medical Faculty Mannheim, Heidelberg University, Mannheim, Germany

[19] Clinical Cooperation Unit Healthy Metabolism, Center for Preventive Medicine and Digital Health Baden-Württemberg (CPDBW), Medical Faculty Mannheim, Heidelberg University, Mannheim, Germany

[20] Department of Internal Medicine II, Division of Hepatology, University Medical Center Mannheim, Medical Faculty Mannheim, Heidelberg University, Mannheim, Germany

[21] Department of Medical Oncology, Oncology Institute of Southern Switzerland, Bellinzona, Switzerland; Faculty of Biomedical Sciences, Università della Svizzera italiana (USI), Lugano, Switzerland

[22] Department of Surgery & Cancer, Imperial College London, Hammersmith Hospital, Du Cane Road, W120HS London, UK

[23] Department of Translational Medicine, Università degli Studi del Piemonte Orientale, Novara, Italy.

[24] Medical Oncology and Hematology Unit, Humanitas Cancer Center, IRCCS Humanitas Research Hospital, Via Manzoni 56, 20089 Rozzano (Milan), Italy

[25] Vienna Hepatic Hemodynamic Laboratory, Medical University of Vienna, Vienna, Austria

[26] Christian-Doppler Laboratory for Portal Hypertension and Liver Fibrosis, Medical University of Vienna, Vienna, Austria

[27] Ludwig Boltzmann Institute for Rare and Undiagnosed Diseases, Vienna, Austria

[28] CeMM Research Center for Molecular Medicine of the Austrian Academy of Sciences, Vienna, Austria

[29] Department of Biomedical Sciences, Humanitas University, Via Rita Levi Montalcini 4, 20090 Pieve Emanuele (Milan), Italy

[30] Cancer Center Esslingen, Klinikum Esslingen, 73730 Esslingen am Neckar, Germany

Correspondence to:

Matthias Pinter, MD PhD

Division of Gastroenterology and Hepatology

Department of Internal Medicine III

Medical University of Vienna

Währinger Gürtel 18-20, 1090 Vienna, Austria

P: +43 1 40400 47440, F: +43 1 40400 47350

M: matthias.pinter@meduniwien.ac.at

Potential conflicts of interest:

B.S. received travel support from AbbVie, Ipsen and Gilead.

K.P. nothing to disclose.

M.K. received honoraria from BMS and AstraZeneca as consultant and is an investigator for AstraZeneca.

F.H. received travel support from Bayer, Abbvie, and Gilead.

F.F. received travel support from Abbvie and Novartis, and speaker fees from Abbvie and MSD.

O.W. served as consultant for Amgen, Bayer, BMS, Celgene, Eisai, Merck, Novartis, Roche, Servier, and Shire. He served as a speaker for Abbvie, Bayer, BMS, Celgene, Falk, Ipsen, Novartis, Roche, and Shire. He received travel support from Abbvie, BMS, Ipsen, Novartis, and Servier.

V.H. has nothing to disclose.

K.S. Served as consultant for Ipsen and Bayer, and conducts studies for Bayer, Roche, Lilly, MSD, and BMS.

J.v.F. has received advisory board fees from Roche.

T.W.F. has nothing to disclose.

M.S. has nothing to disclose.

H.H. received compensations as a member of a scientific advisory board of Lilly.

K.S. has nothing to disclose.

S.S. has nothing to disclose.

P.R. has nothing to disclose.

A.R.S. has served at advisory boards and received consulting honoraria from AMGEN, AAA, Bayer, BMS, IPSEN, Lilly, Merck, MSD, Pfizer, Roche, Sanofi, and Servier.

J.C.M. has received consulting honoraria from Abbvie, Astra-Zeneca, Bayer, BMS, Eisai, Gilead, Incyte, Intercept, MSD, Sanofi, Vifor for work performed outside the current study

N.N.R. has nothing to disclose.

F.K. received speakers' fees from Bayer, Ipsen, MSD, Eisai, Shire, Sirtex and has received travel grants from Eisai, Janssen, Ipsen and Novartis.

D.T.W. served as speaker/expert testimony for AstraZeneca, Eisai, BMS, Celgene, Incyte, Ipsen, Falk, Novartis, Roche Pharma AG, Servier, Shire Baxelta, and Sirtex; he received travel support from Bayer Health Pharma, Celgene, Ipsen, Novartis, and SIRTEX, and research grants/funding from Servier.

M.P.E. received consulting honoraria from BMS and MSD.

A.T. received consulting honoraria and /or lecture fees from Bayer, IPSEN, Lilly, BMS, Eisai Novartis, Roche, Intercept, Falk, AbbVie, and Gilead. He received and travel grants from IPSEN, AbbVie, and Gilead. He is an investigator for IPSEN and GILEAD.

S.D.D. received consulting honoraria from Amgen, Bayer, BMS, IPSEN, Lilly, Merck, BMS, Novartis, Pfizer, Roche, Sanofi, and Servier, and travel grants from Amgen, BMS, IPSEN, Roche, and Servier.

D.J.P. received lecture fees from ViiV Healthcare, Bayer Healthcare, Falk, BMS, EISAI and Roche; travel expenses from BMS, MSD and Bayer Healthcare; consulting fees for Mina Therapeutics, EISAI, H3B, Roche, Astra Zeneca, and DaVolterra; research funding (to institution) from MSD, BMS.

T.P. received consulting fees from IQVIA and Bayer; and institutional research funding from Lilly, Roche, Bayer.

T.M. has nothing to disclose.

L.B. has nothing to disclose.

C.M. has nothing to disclose.

M.M. served as a speaker and/or consultant and/or advisory board member for AbbVie, Bristol-Myers Squibb, Collective Acumen, Gilead, and W. L. Gore & Associates and received travel support from AbbVie, Bristol-Myers Squibb, and Gilead.

T.R. received speaker fees from Boehringer Ingelheim, Roche, W.L. Gore and MSD, grant support from Boehringer Ingelheim, Boston Scientific, Cook Medical, Gilead, Guerbet, Abbvie, Phenex Pharmaceuticals, Philips, W.L. Gore, and MSD, served as a consultant for Abbvie, Bayer, Boehringer Ingelheim, Gilead, Intercept and MSD and received travel support from Gilead, Roche, MSD, and Gore.

M.T. received speaker fees from Bristol-Myers Squibb (BMS), Falk Foundation, Gilead, Intercept and Merck Sharp & Dohme (MSD); advisory board fees from Abbvie, Albireo, Boehringer Ingelheim, BiomX, Falk Pharma GmbH, GENFIT, Gilead, Intercept, Janssen, MSD, Novartis, Phenex, Regulus and Shire; travel grants from AbbVie, Falk, Gilead, and Intercept; and research grants from Albireo, CymaBay, Falk, Gilead, Intercept, MSD, and Takeda. He is also coinventor of patents on the medical use of norUDCA filed by the Medical University of Graz.

N.P. received consulting fees from Amgen, Merck Serono, Servier; lectures fees from AbbVie, Gilead, Lilly; travel fees from Amgen, ArQule; and institutional research funding from Basilea, Merck Serono, Servier.

L.R. has received consulting fees from Amgen, ArQule, AstraZeneca, Basilea, Bayer, BMS, Celgene, Eisai, Exelixis, Genenta, Hengrui, Incyte, Ipsen, IQVIA, Lilly, MSD, Nerviano Medical Sciences, Roche, Sanofi, Zymeworks; lectures fees from AbbVie, Amgen, Bayer, Eisai, Gilead, Incyte, Ipsen, Lilly, Merck Serono, Roche, Sanofi; travel expenses from Ipsen; and institutional research funding from Agios, ARMO BioSciences, AstraZeneca, BeiGene, Eisai, Exelixis, Fibrogen, Incyte, Ipsen, Lilly, MSD, Nerviano Medical Sciences, Roche, Zymeworks.

M.B. received compensations as a member of scientific advisory boards of Bayer, Bristol–Meyers Squibb, EISAI, IPSEN, and MSD.

J.T. served as consultant for Amgen, Bayer, BMS, Eisai, Lilly, Merck Serono, MSD, Ipsen, and Roche, received travel support from BMS and Ipsen, and speaking fees from Amgen, Bayer, BMS, Eisai, Lilly, Merck Serono, MSD, Ipsen, and Roche. He is also an investigator for Amgen, Bayer, BMS, Eisai, Lilly, Merck Serono, MSD, Ipsen, and Roche.

A.W. received compensations as a member of scientific advisory boards for BMS, Wako, Sanofi and. He served as a speaker for Leo Pharma, Eisai, Ipsen and Roche and received travel support from Merck and Servier.

H.W. served as speaker for Bayer, Eisai, Ipsen, and Roche, and as a consultant for Bayer, Eisai, Lilly, BMS, Roche, and Ipsen. He conducts studies for Bayer, Roche, Lilly, MSD, and BMS.

J.F.D. received compensations as a member of scientific advisory boards of Abbvie, Bayer, Bristol–Myers Squibb, Falk, Galapagos, Genfit, Genkyotex, Gilead Sciences, HepaRegenix, Intercept, Lilly, Merck, and Novartis.

M.P.R. is advisor/consultant for Astra Zeneca, Bayer, BMS, Eisai, Ipsen, Lilly, MSD, and Roche; he served as a speaker for Bayer, Eisai, Ipsen, Lilly, and Roche; he is an investigator for Bayer, BMS, Eisai, Exelixis, Lilly, and Roche.

A.V. served as consultant for Roche, Bayer, Lilly, BMS, Eisai, and Ipsen, and received speaking fees form Roche, Bayer, Lilly, BMS, Eisai, and Ipsen. He is also an investigator for Roche, Bayer, Lilly, BMS, Eisai, and Ipsen.

M.P. is an investigator for Bayer, BMS, Lilly, and Roche, he received speaker honoraria from Bayer, BMS, Eisai, and MSD, he is a consultant for Bayer, BMS, Ipsen, Eisai, Lilly, Roche, and MSD, and he received travel support from Bayer, BMS, and Roche.

Acknowledgements

Funding: D.J.P. acknowledges the infrastructural support provided by Imperial Experimental Cancer Medicine Centre, Cancer Research UK Imperial Centre, the Imperial College BRC and the Imperial College Healthcare NHS Trust Tissue Bank.

Author contributions:

All authors contributed either to research design (B.S. and M.P.), and/or the data acquisition (B.S., K.P. M.M.K., F.H., F.F., O.W., V.H., K.S. J.v.F., T.W.F., K.S., S.S., P.R., A.R.S., J.C.M, N.N.R, F.K, D.-T.W., M.P.E., A.T, S.D.D., T.P., T.M., L.P., C.M., M.M., T.R., M.T., N.P., L.R., M.B., J.T., A.W., H.W., J.-F.D., M.P.-R., A.V., M.P.), analysis (B.S., M.S., H.H., and M.P.), or interpretation (all authors) of data. B.S., H.H., and M.P. drafted the manuscript, which was critically revised by all other authors.

Abstract

Background: Immunotherapy with atezolizumab plus bevacizumab represents the new standard of care in systemic front-line treatment of hepatocellular carcinoma (HCC). Prognostic biomarkers are an unmet need.

Methods: Patients with HCC put on PD-(L)1-based immunotherapy in 6 European centers (training set; n=190) and in 8 European centers (validation set; n=102) were included. We investigated the prognostic value of baseline variables on overall survival by using a Cox model in the training set and developed the easily applicable CRAFITY (**CR**P and **AF**P in **I**mmuno**T**herap**Y**) score. The score was validated in the independent, external cohort, and evaluated in a cohort of patients treated with sorafenib (n=204).

Results: Baseline serum alpha-fetoprotein ≥100 ng/ml (HR, 1.7; p=0.007) and C-reactive protein ≥1 mg/dl (HR, 1.7; p=0.007) were identified as independent prognostic factors in multivariable analysis and were used to develop the CRAFITY score. Patients who fulfilled no criterion (0 points; CRAFITY-low) had the longest median overall survival (27.6 (95%CI, 19.5-35.8) months), followed by those fulfilling one criterion (1 point; CRAFITY-intermediate; 11.3 (95%CI, 8.0-14.6) months), and patients meeting both criteria (2 points; CRAFITY-high; 6.4 (95%CI, 4.8-8.1) months; p<0.001). Additionally, best radiological response (complete response/partial response/stable disease/progressive disease) was significantly better in patients with lower CRAFITY score (CRAFITY-low:9%/20%/52%/20% vs. CRAFITY-intermediate:3%/25%/36%/36% vs. CRAFITY-high:2%/15%/22%/61%; p=0.003). These results were confirmed in the independent validation set as well as in different subgroups including Child-Pugh A and B, performance status 0 and ≥1, and first-line

and later lines. In the sorafenib cohort, CRAFITY was associated with survival, but not radiological response.

Conclusions: The CRAFITY score is associated with survival and radiological response. The score may help with patient counseling, but requires prospective validation.

Key words: C-reactive protein, alpha-fetoprotein, immune checkpoint inhibitor, liver cancer

Lay summary:

The immunotherapy based regimen of atezolizumab plus bevacizumab represents the new standard of care in systemic first-line therapy of hepatocellular carcinoma (HCC). Biomarkers to predict treatment outcome are an unmet need in patients undergoing immunotherapy for HCC. We developed and externally validated a score that predicts outcome in patients with HCC undergoing immunotherapy with immune checkpoint blockers.

Introduction

Hepatocellular carcinoma (HCC) is the most common primary liver cancer and usually develops in patients with underlying liver disease [1]. With sorafenib representing the only available systemic therapy for roughly a decade, the treatment landscape has expanded rapidly over the last few years. Beside several tyrosine kinase inhibitors and a monoclonal antibody against VEGFR-2 being added to the treatment armamentarium, immunotherapy with immune checkpoint blockers (ICBs) has been extensively investigated in patients with HCC [2-4]. Nivolumab, pembrolizumab, and the combination of nivolumab plus ipilimumab have been approved by the United States Food And Drug Administration (FDA) based on phase II studies [4], but nivolumab and pembrolizumab failed to reach their primary endpoints in subsequent phase III trials in first- and second-line, respectively [5, 6]. The combination of atezolizumab with bevacizumab finally succeeded in a phase III trial versus sorafenib [7], making this combination the new reference standard in front-line systemic treatment for the majority of HCC patients [8].

Approximately one-third of patients achieved an objective response with atezolizumab plus bevacizumab [7], which is almost twice as high as compared to PD-1 monotherapy [5, 6]. Several predictive biomarkers for immunotherapy have been proposed, including PD-L1 expression [9, 10] and activated Wnt/β-catenin signaling [11], but currently no validated biomarker exists to guide treatment decisions in HCC patients undergoing immunotherapy. Exploratory analyses of the CheckMate 040 study demonstrated that an inflammatory gene signature was associated with response and survival in nivolumab-treated HCC patients [9], suggesting that inflammatory biomarkers could aid in the identification of patients who benefit from immunotherapy.

In the current study, we developed a simple and easily applicable score to predict treatment success (defined as response or disease stabilization) as well as survival in patients with HCC undergoing immunotherapy with ICBs, which was validated in an independent, external cohort.

Patients and methods

Study design and patients

Immunotherapy cohorts:

Patients with histologically or radiologically diagnosed HCC who received anti-PD-(L)1-based immunotherapy were considered for this retrospective study. Patients who received immunotherapy in combination with loco-regional therapies or as adjuvant treatment after curative therapies were excluded. Only patients with available baseline serum alpha-fetoprotein (AFP) and C-reactive protein (CRP) levels were eligible. The training set included patients from 6 centers in Austria and Germany that participated in a previous publication on immunotherapy in HCC [12]. In these patients, PD-(L)1-targeted immunotherapy was initiated between July 2015 and December 2020. The validation set included patients from 8 centers in Germany, Italy, and Switzerland in whom anti-PD-(L)1-based immunotherapy was initiated between August 2015 and December 2020. Data from some of these centers were published previously [12-14]. The training and the validation set represent patient populations treated within a similar therapeutic setting and thus, these cohorts are expected to be inherently comparable in terms of patient and tumor characteristics.

Sorafenib cohort:

Patients with HCC in whom sorafenib was initiated between May 2006 and April 2019 at the Medical University of Vienna were included as a non-immunotherapy cohort. Patients who received combination treatments (e.g., with local ablative therapy/chemoembolization/SIRT) and patients with insufficient medical records were excluded. We also excluded patients who received prior or subsequent immunotherapy. Similar to the immunotherapy cohorts, only patients with available baseline serum AFP and CRP were eligible.

In all cohorts, data including patient history, laboratory results, and radiological information were collected retrospectively. The start of immunotherapy or sorafenib was considered the baseline. The retrospective analysis was approved by the Ethics Committee of the Medical University of Vienna. Informed consent was waived due to the retrospective nature of the study.

Assessments

Computed tomography (CT) and/or magnetic resonance imaging (MRI) were performed at baseline and about every 6 to 12 weeks thereafter in both the training and validation set. Patients who had at least one radiological follow-up imaging were evaluated for best radiological response, which was evaluated according to the modified Response Evaluation Criteria in Solid Tumors (mRECIST) [15]. Disease control rate (DCR) was defined as the proportion of patients achieving complete/partial response or stable disease as best radiological response. Laboratory values were included if obtained at least within 1 month before immunotherapy initiation. If more than one value was available, the closest to treatment start was chosen; this was frequently but not always the value obtained at day one of cycle 1.

Statistics

As this is a retrospective study, no formal sample size calculation was performed, instead, all available patients fulfilling in- and exclusion criteria were considered for this study.

Data on baseline characteristics and radiological tumor response were summarized using descriptive statistics. Chi square test or Fisher's exact test were used to compare nominal data. Standardized differences were calculated to compare patient and tumor characteristics between different cohorts [16]. Overall survival (OS) was defined as the time from start of immunotherapy/sorafenib until death, and patients who were still alive were censored at the date of last contact. Survival curves were calculated by the Kaplan-Meier method and compared by means of the log rank test.

Uni- and multivariable analyses were conducted with Cox regression models. Variables with a p-value <0.05 on univariable analysis were considered for multivariable analysis. In order to develop an easy-to-apply score, which provides additional prognostic insight to already well-established factors, the following strategy was chosen. At first, the functional forms of the effects of continuous variables (in concrete terms, CRP and AFP) on OS was flexibly assessed with restricted cubic splines [17]. Based on these results, easy-to-remember cut-off values for AFP and CRP were chosen which are also compatible with previous publications [18-23]. To avoid overoptimistic results by developing and testing the score in the same dataset, we validated the score in an independent, external validation cohort [24, 25].

Internal validation of the model was performed via the bootstrap method.

As overall discrimination measure, the c-statistic for the Cox model by Uno was applied [26]. We also performed time-dependent area under the receiver operating characteristic curve analyses and reported the corresponding AUC-values at 12, 24,

and 36 months (30 months in validation set). Further details on statistical methods for model validation are specified in the supplement, where also some additional results can be found.

To assess the potential effect of missing values on radiological tumor response, a worst case sensitivity analysis was performed, that is, all missing values were considered as disease progression.

Median estimated follow-up was calculated using the reverse Kaplan-Meier method [27].

Statistical analyses were performed using IBM SPSS Statistics version 26 (SPSS Inc., Chicago, IL), SAS 9.4 (SAS Institute Inc., Cary, NC) and GraphPad Prism 8 (GraphPad Software, Inc., San Diego, CA). A two-sided significance level of 5 percent was used.

Results

Patient characteristics (training set)

One-hundred and ninety patients receiving anti-PD-(L)1-based immunotherapy were included in the training set (Figure 1). Baseline characteristics are described in Table 1 and type of immunotherapeutic agents used are shown in Supplemental Table 1. Immunotherapy was used as systemic first-, second-, or later-line treatment in 82 (43%), 57 (30%), and 51 (27%) patients, respectively. The majority (78%) had advanced stage HCC and Child-Pugh class A (53%). Median duration of estimated follow-up was 15.6 (95%CI, 4.2-27.1) months in the whole cohort, and 9.1 (95%CI, 6.3-11.9) months in first-line patients, 30.8 (95%CI, 24.1-37.6) months in second-line, and 31.2 (95%CI, 26.3-36.2) months in later lines.

Efficacy (training set)

Median OS was 11.0 (95%CI, 7.1-14.8) months. In univariable analysis, Child-Pugh stage, performance status, AFP, and CRP were significantly associated with OS (Table 2) and included in a multivariable model. Beside Child-Pugh stage and performance status, AFP <100 vs. ≥100 ng/ml (HR, 1.7 (95%CI 1.2-2.6) and CRP <1 vs. ≥1 mg/dl (HR, 1.7 (95%CI 1.2-2.6) remained independent prognostic factors after multivariable adjustment (Table 2, Supplemental Table 2).

One-hundred and fifty-six (82%) patients with at least one follow-up imaging were available for radiological tumor response assessment. Overall, seven of them (5%) achieved complete response (CR) and 32 (21%) subjects had partial response (PR), resulting in an ORR of 25%. Fifty-seven (37%) patients had stable disease (SD) and 60 (39%) progressed (progressive disease, PD) at first radiological assessment.

In patients receiving immunotherapy as first-line, 2 (3%)/16 (24%)/24 (36%)/24 (36%) had CR/PR/SD/PD compared to 4 (8%)/9 (18%)/17 (34%)/20 (40%) patients treated in second-line and 1 (3%)/7 (18%)/16 (40%)/17 (40%) patients undergoing immunotherapy in later lines. The disease control rate (DCR) was 62% in the whole cohort, and 64%, 60%, and 60% in patients treated in first-, second- and later lines, respectively.

The CRAFITY score predicts overall survival in HCC patients undergoing PD-(L)1-targeted immunotherapy (training set)

Next, we aimed to develop an objective, lab-based score to predict outcome of HCC patients undergoing immunotherapy. Given that both AFP and CRP were prognostic factors, independently of Child-Pugh class and ECOG performance status, and that both had similar regression coefficients (both 0.55) in multivariable analysis, we

developed a simple score based on those two variables and assigned 1 point for having an AFP ≥100 ng/ml and 1 point for having a CRP ≥1 mg/dl. Thus, a patient could achieve either 0 (AFP <100 ng/ml and CRP <1 mg/dl), 1 (either AFP ≥100 ng/ml or CRP ≥1 mg/dl), or 2 (AFP ≥100 ng/ml and CRP ≥1 mg/dl) points. The resulting score was named CRAFITY score (**CR**P and **AF**P in **I**mmuno**T**herap**Y**). Restricted cubic spline analyses supported the cut-offs for CRP and AFP (Figure 2).

Median OS of patients with 0 points (CRAFITY-low, n=53), 1 point (CRAFITY-intermediate, n=75), and 2 points (CRAFITY-high, n=62) was 27.6 (95%CI, 19.5-35.8) months, 11.3 (95%CI, 8.0-14.6) months, and 6.4 (95%CI, 4.8-8.1) months (p<0.001) (Figure 3A).

The CRAFITY score predicts radiological response in HCC patients undergoing PD-(L)1-targeted immunotherapy (training set)

In patients with at least one follow-up imaging (n=156), CRAFITY score correlated with better best radiological response, as CR was n= 4/46 (9%) vs. n= 2/64 (3%) vs. n= 1/46 (2%), and PR was n= 9/46 (20%) vs. n= 16/64 (25%) vs. n=7/46 (15%) for CRAFITY-low vs. CRAFITY-intermediate vs. CRAFITY-high; SD was n=24/46 (52%) vs. n=23/64 (36%) vs. n=10/46 (22%), and PD was n=9/46 (20%) vs. n=23/64 (36%) vs. n=28/46 (61%) for CRAFITY-low vs. CRAFITY-intermediate vs. CRAFITY-high (p=0.001). The DCR was 80% vs. 64% vs. 39% for CRAFITY-low vs. CRAFITY-intermediate vs. CRAFITY-high (p<0.001) (Table 3).

Given that radiological follow-up was not available in 18% of patients, we performed a worst case scenario analysis assigning all patients without radiological evaluation to the progressive disease group. While the DCR was lower in all CRAFITY subgroups, the difference between the subgroups remained unchanged (Supplemental Table 3).

The CRAFITY score predicts overall survival in an independent external cohort of HCC patients undergoing PD-(L)1-targeted immunotherapy (validation set)

We next validated our results in an independent cohort of 102 patients treated with immunotherapy (Figure 1). Baseline characteristics are described in Table 1 and type of immunotherapeutic agents used are shown in Supplemental Table 1. Median duration of estimated follow-up was 24.8 (95%CI, 17.4-32.1) months in the overall cohort, and 9.2 (95%CI, 5.7-12.8) months in first-line, 31.3 (95%CI, 24.5-38.0) months in second-line, and 25.8 (95%CI, 25.3-26.3) months in later lines. Median OS was 13.9 (95%CI, 10.1-17.7) months in the whole cohort, and 21.7 (95%CI, 5.5-38.0) months for CRAFITY-low (n=26), 13.9 (95%CI, 7.4-20.4) months for CRAFITY-intermediate (n=47), and 8.4 (95%CI, 6.6-10.1) months for CRAFITY-high (n=29) (p=0.001) (Figure 3B).

The CRAFITY score is associated with radiological response in an independent external cohort of HCC patients undergoing PD-(L)1-targeted immunotherapy (validation set)

Ninety (88%) patients had at least one follow-up imaging and were therefore available for radiological response evaluation (CRAFITY-low vs. CRAFITY-intermediate vs. CRAFITY-high, n=25/26 vs. n=41/47 vs. n= 24/29). The DCR was n=20/25 (80%) vs. n=28/41 (68%) vs. 11/24 (46%) for CRAFITY-low vs. CRAFITY-intermediate vs. CRAFITY-high (p=0.037).

Score validation

C-statistics for the CRAFITY score in the training as well as in the validation set were 0.62 each. Using time-dependent AUCs, discrimination in the training set was 0.71 (95%CI: 0.62-0.80), 0.69 (95%CI: 0.59-0.80), and 0.62 (95%CI: 0.40-0.84) at 12, 24, and 36 months. Results of time-dependent AUCs were comparable for the validation set (Supplemental Figure 1, Supplemental Material).

When comparing the Cox-model fit with Kaplan-Meier plots, good agreement (calibration) between the predictions from the model to the observed survival probabilities was observed; the respective figures for the training as well as the validation set are displayed in the Supplemental material (Supplemental Figure 2). Further information on the overall performance of the CRAFITY score in the training as well as the validation set can be found in the Supplemental material.

Internal bootstrap validation generated consistent results and confirmed the overall performance (Supplemental material).

The CRAFITY score predicts outcome in different subgroups (pooled set)

In order to increase the number of patients for exploratory subgroup analyses, we pooled the training and validation set (n=292).

In the pooled cohort, the median OS was 27.5 (95%CI, 16.9-38.2) months for CRAFITY-low (n=79), 13.5 (95%CI, 11.0-16.1) months for CRAFITY-intermediate (n=122), and 6.9 (95%CI, 5.4-8.4) months for CRAFITY-high (n=91) (Figure 3C).

In Child-Pugh A patients, median OS was 31.3 (95%CI, 20.7-41.8) months for CRAFITY-low (n=54), 21.5 (95%CI, 5.0-38.0) months for CRAFITY-intermediate (n=75), and 11.0 (95%CI, 0.0-21.9) months for CRAFITY-high (n=44) ($p<0.001$). Similarly, in Child-Pugh B patients, median OS was 21.8 (95%CI, 15.1-28.5) months

for CRAFITY-low (n=23), 7.6 (95%CI, 1.9-13.4) months for CRAFITY-intermediate (n=40), and 6.4 (95%CI, 4.3-8.6) months for CRAFITY-high (n=37) (p=0.003).

In patients who received immunotherapy as first-line, median OS was 27.6 (95%CI, 11.7-43.6) months for CRAFITY-low (n=34), 11.1 (95%CI, 2.4-19.9) months for CRAFITY-intermediate (n=52), and 4.8 (95%CI, 2.5-7.1) months for CRAFITY-high (n=31) (p<0.001). In patients who underwent immunotherapy in second-line, median OS was 31.3 (95%CI, 17.4-45.2) months for CRAFITY-low (n=25), 13.9 (95%CI, 7.7-20.2) months for CRAFITY-intermediate (n=46), and 6.8 (95%CI, 2.3-11.3) months for CRAFITY-high (n=30) (p<0.001). Similarly, in patients treated with immunotherapy in later lines, median OS was 19.6 (95%CI, 10.6-28.6) months for CRAFITY-low (n=20), 13.4 (95%CI, 4.6-22.3) months for CRAFITY-intermediate (n=24), and 8.6 (95%CI, 7.4-9.8) months for CRAFITY-high (n=30) (p=0.036).

Comparable results were also obtained in further subgroups including patients with viral and non-viral etiology, age <65 and ≥65 years, ECOG PS 0 and ≥1, absence and presence of macrovascular invasion, and absence and presence of extrahepatic metastases (Figure 4, Supplemental Table 4 and 5). Two-hundred and forty-six (84%) patients were available for assessment of radiological response. Disease control rates for different subgroups are displayed in Supplementary Table 4. Results of 20 patients with BCLC stage D are shown in the Supplementary Results.

Performance of CRAFITY score in a sorafenib-treated cohort of patients with HCC

We next investigated the CRAFITY score in an independent cohort of 204 patients in whom sorafenib was initiated between May 2006 and April 2019. Baseline characteristics are described in Supplemental Table 6.

Median duration of estimated follow-up was 58.9 (95%CI, 39.9-77.9) months and median OS was 8.2 (95%CI, 6.5-10.0) months. One-hundred and forty-one (69%) patients had at least one follow-up imaging and were therefore available for radiological response assessment.

While the CRAFITY score was associated with survival, it failed to predict DCR in sorafenib-treated patients. Median OS was 18.3 (95%CI, 12.2-24.3) months for CRAFITY-low (n=47), 7.9 (95%CI, 6.1-9.7) months for CRAFITY-intermediate (n=90), and 4.6 (95%CI, 3.0-6.1) months for CRAFITY-high (n=67) ($p<0.001$) (Figure 3D). The DCR was n=13/35 (37%) vs. n=40/65 (62%) vs. 15/41 (37%) for CRAFITY-low vs. CRAFITY-intermediate vs. CRAFITY-high.

Direct comparison between patients with BCLC A-C treated with sorafenib or immunotherapy in systemic first-line revealed that median OS was longer and DCR was higher in immunotherapy-treated patients within the CRAFITY-low/intermediate group, while there was no substantial difference between sorafenib- and immunotherapy-treated patients within CRAFITY-high (details are outlined in the Supplemental results and Supplemental Table 7).

Discussion

In the current work, we developed a simple, easily applicable score – based on the two serum parameters AFP and CRP – that predicts likelihood of immunotherapy success and improved survival in patients with advanced HCC who received ICBs. Accordingly, in contrast to patients with an AFP of 100ng/mL or higher and a CRP of 1mg/dL or higher, patients who fulfilled none of these criteria had an excellent disease control rate and survival; patients who fulfilled only one criterion still had an improved outcome. These results were confirmed in an independent, external validation cohort

of HCC patients treated with ICBs. While the score was also prognostic in HCC patients treated with sorafenib, it was not able to predict DCR.

Both AFP and CRP are well known prognostic factors in HCC and have been incorporated in different prognostic models [18, 19, 28-32]. Notably, our score was not only associated with survival but also with achieving radiological disease control (response or stabilization) with ICBs. Achieving disease stabilization or response can be considered a treatment success since both are associated with improved survival rates compared to patients having progressive disease as best radiological response [33, 34]. In our study, progressive disease was observed in 39% of patients at first radiological assessment which is comparable to phase III studies testing PD-1-targeted monotherapy in first- (37%)[6] or second-line (32%)[5], but higher compared to the IMbrave150 trial testing the combination of atezolizumab and bevacizumab (20%) [34]. This might be due to the fact that the majority of our patients received anti-PD1-monotherapy in second- or later lines.

There is a good rationale for the combined use of AFP and CRP to predict outcome of patients with HCC undergoing immunotherapy. Inflammation – a hallmark of cancer – contributes to tumorigenesis and cancer progression [35]. CRP is an acute phase protein and a well-accepted marker of cancer-induced systemic inflammation, a condition which is clinically often reflected in cancer symptoms such as anorexia, weight loss, and fatigue [36-38]. In the local tumor microenvironment, inflammation has several tumor-promoting effects, including fostering of cancer cell proliferation, metastatic seeding, angiogenesis, as well as inhibition of adaptive immunity [39]. CRP has also been directly linked to tumor progression. In myeloma studies, CRP enhanced cell proliferation and prevented chemotherapy-induced apoptosis [40]. Moreover, interleukin-6 – a main inducer of CRP – is associated with

hepatocarcinogenesis and development of liver metastases from other cancer types [41-43].

More recent evidence links CRP to tumor immunosuppression. CRP suppresses proliferation and effector functions of activated CD4+ and CD8+ T cells from melanoma patients, reduces the expression of co-stimulatory signals on mature dendritic cells (DC), and inhibits the expansion of MART-1 antigen specific CD8+ T cells [44]. CRP also promotes expansion of myeloid derived suppressor cells [45]. In lung cancer patients, high CRP was associated with PD-L1 positivity [46].

By exerting these immunosuppressive effects, CRP may impair the efficacy of immunotherapy. Indeed, several studies reported that elevated baseline CRP levels were associated with reduced response rates and/or shorter survival in ICB-treated patients with different tumor types, including non-small cell lung cancer and melanoma [44, 47-49].

AFP is a widely used serum biomarker in the management of HCC and the only biomarker to guide treatment decisions in HCC [20, 50]. Beside promoting tumor growth, partly by inhibition of apoptosis [20, 51], AFP may also hinder anti-tumor immunity as it suppresses proliferation of T lymphocytes, inhibits natural killer cell activity and DC differentiation, and increases the activity of T suppressor cells [20, 52, 53].

Moreover, emerging evidence suggests that AFP is also associated with up-regulation of VEGF signaling [54, 55]. VEGF is not only a main regulator of angiogenesis but also fosters an immunosuppressive tumor microenvironment by inhibiting function and maturation of effector T cells and antigen-presenting cells, promoting infiltration of immunosuppressive cell types, and up-regulating immune checkpoint molecules (i.e., PD-1) [4, 56]. Together, these data indicate that both CRP and AFP affect tumor cells

either directly or indirectly via modulation of the tumor microenvironment and promote an immunosuppressive milieu that may hamper the efficacy of immunotherapy.

We want to acknowledge the retrospective design as a limitation of the study, which prevented scheduled radiological assessment. Thus, to ultimately evaluate the CRAFITY score as a predictor of radiological response, a prospective cohort with predefined and homogeneous imaging assessments is required. The CRAFITY score was developed in patients with mainly intermediate-advanced HCC not amenable to surgical or loco-regional therapies, however the cohort was heterogeneous in terms of liver function, treatment line, and type of immunotherapy potentially leading to a selection bias. For instance, some patients received ICBs in third or even later lines of systemic treatment which could reflect selection of tumors with a less aggressive tumor biology. Moreover, some patients had advanced or decompensated liver cirrhosis who, by nature, have a poorer outcome and thus, are usually excluded from clinical trials. Nevertheless, they are often treated in real-life practice. To account for a potential selection bias, we included these and other relevant variables as candidate covariates in our multivariable modelling strategy and successfully assessed our score in several subgroups of interest. Additionally, CRP is associated with increased cardiovascular events [57], which may represent a competing risk, especially in patients with metabolic-associated liver disease. Even though we demonstrated that the CRAFITY score was only prognostic for OS but not predictive for DCR in the sorafenib group, these analyses do not replace a true control group. Finally, in phase III randomized controlled immunotherapy trials [5-7], AFP was not associated with a lack of immunotherapy efficacy versus control group. Our data suggest that high AFP and CRP are associated with worse outcome, however, this does not equal lack of

efficacy. Therefore, immunotherapy should not be withheld just because of high AFP or CRP.

In conclusion, we developed an externally validated score combining AFP and CRP – both known to promote immunosuppression – that predicts outcome of patients undergoing immunotherapy for HCC, independently of Child-Pugh class and performance status. Since the CRAFITY score is based on two lab values ubiquitously available, it is objective and widely applicable. The score could aid in the selection of patients for clinical trial inclusion and support decision-making in daily clinical practice. The score warrants prospective validation in a large clinical study, ideally with an active control not treated with immunotherapy.

References

[1] Pinter M, Trauner M, Peck-Radosavljevic M, Sieghart W. Cancer and liver cirrhosis: implications on prognosis and management. ESMO Open 2016;1:e000042.
[2] European Association for the Study of the Liver. Electronic address eee, European Association for the Study of the L. EASL Clinical Practice Guidelines: Management of hepatocellular carcinoma. J Hepatol 2018;69:182-236.
[3] Pinter M, Peck-Radosavljevic M. Review article: systemic treatment of hepatocellular carcinoma. Aliment Pharmacol Ther 2018;48:598-609.
[4] Pinter M, Jain RK, Duda DG. The Current Landscape of Immune Checkpoint Blockade in Hepatocellular Carcinoma: A Review. JAMA Oncol 2020.
[5] Finn RS, Ryoo BY, Merle P, Kudo M, Bouattour M, Lim HY, et al. Pembrolizumab As Second-Line Therapy in Patients With Advanced Hepatocellular Carcinoma in KEYNOTE-240: A Randomized, Double-Blind, Phase III Trial. J Clin Oncol 2020;38:193-202.
[6] Yau T, Park JW, Finn RS, Cheng A, Mathurin P, Edeline J, et al. CheckMate 459: A Randomized, Multi-Center Phase 3 Study of Nivolumab (NIVO) vs Sorafenib (SOR) as First-Line (1L) Treatment in Patients (pts) With Advanced Hepatocellular Carcinoma. Ann Oncol 2019;30 (suppl_5):v874-v875.
[7] Finn RS, Qin S, Ikeda M, Galle PR, Ducreux M, Kim TY, et al. Atezolizumab plus Bevacizumab in Unresectable Hepatocellular Carcinoma. N Engl J Med 2020;382:1894-1905.
[8] Pinter M, Scheiner B, Peck-Radosavljevic M. Immunotherapy for advanced hepatocellular carcinoma: a focus on special subgroups. Gut 2021;70:204-214.
[9] Sangro B, Melero I, Wadhawan S, Finn RS, Abou-Alfa GK, Cheng AL, et al. Association of inflammatory biomarkers with clinical outcomes in nivolumab-treated patients with advanced hepatocellular carcinoma. J Hepatol 2020;73:1460-1469.
[10] Zhu AX, Finn RS, Edeline J, Cattan S, Ogasawara S, Palmer D, et al. Pembrolizumab in patients with advanced hepatocellular carcinoma previously treated with sorafenib (KEYNOTE-224): a non-randomised, open-label phase 2 trial. Lancet Oncol 2018;19:940-952.
[11] Sia D, Jiao Y, Martinez-Quetglas I, Kuchuk O, Villacorta-Martin C, Castro de Moura M, et al. Identification of an Immune-specific Class of Hepatocellular Carcinoma, Based on Molecular Features. Gastroenterology 2017;153:812-826.
[12] Scheiner B, Kirstein MM, Hucke F, Finkelmeier F, Schulze K, von Felden J, et al. Programmed cell death protein-1 (PD-1)-targeted immunotherapy in advanced hepatocellular carcinoma: efficacy and safety data from an international multicentre real-world cohort. Aliment Pharmacol Ther 2019;49:1323-1333.
[13] Spahn S, Roessler D, Pompilia R, Gabernet G, Gladstone BP, Horger M, et al. Clinical and Genetic Tumor Characteristics of Responding and Non-Responding Patients to PD-1 Inhibition in Hepatocellular Carcinoma. Cancers (Basel) 2020;12.
[14] Pfister D, Nunez NG, Pinyol R, Govaere O, Pinter M, Szydlowska M, et al. NASH limits anti-tumour surveillance in immunotherapy-treated HCC. Nature 2021;592:450-456.
[15] Lencioni R, Llovet JM. Modified RECIST (mRECIST) assessment for hepatocellular carcinoma. Semin Liver Dis 2010;30:52-60.
[16] Austin PC. An Introduction to Propensity Score Methods for Reducing the Effects of Confounding in Observational Studies. Multivariate Behav Res 2011;46:399-424.
[17] Heinzl H, Kaider A. Gaining more flexibility in Cox proportional hazards regression models with cubic spline functions. Comput Methods Programs Biomed 1997;54:201-208.

[18] Sieghart W, Pinter M, Hucke F, Graziadei I, Schoniger-Hekele M, Muller C, et al. Single determination of C-reactive protein at the time of diagnosis predicts long-term outcome of patients with hepatocellular carcinoma. Hepatology 2013;57:2224-2234.
[19] Meischl T, Rasoul-Rockenschaub S, Gyori G, Sieghart W, Reiberger T, Trauner M, et al. C-reactive protein is an independent predictor for hepatocellular carcinoma recurrence after liver transplantation. PLoS One 2019;14:e0216677.
[20] Galle PR, Foerster F, Kudo M, Chan SL, Llovet JM, Qin S, et al. Biology and significance of alpha-fetoprotein in hepatocellular carcinoma. Liver Int 2019;39:2214-2229.
[21] Tyson GL, Duan Z, Kramer JR, Davila JA, Richardson PA, El-Serag HB. Level of alpha-fetoprotein predicts mortality among patients with hepatitis C-related hepatocellular carcinoma. Clin Gastroenterol Hepatol 2011;9:989-994.
[22] Hayashi T, Shibata M, Oe S, Miyagawa K, Honma Y, Harada M. C-reactive protein can predict dose intensity, time to treatment failure and overall survival in HCC treated with lenvatinib. PLoS One 2020;15:e0244370.
[23] Takada H, Kurosaki M, Nakanishi H, Takahashi Y, Itakura J, Tsuchiya K, et al. Impact of pre-sarcopenia in sorafenib treatment for advanced hepatocellular carcinoma. PLoS One 2018;13:e0198812.
[24] Steyerberg EW, Vergouwe Y. Towards better clinical prediction models: seven steps for development and an ABCD for validation. Eur Heart J 2014;35:1925-1931.
[25] Steyerberg EW, Vickers AJ, Cook NR, Gerds T, Gonen M, Obuchowski N, et al. Assessing the performance of prediction models: a framework for traditional and novel measures. Epidemiology 2010;21:128-138.
[26] Uno H, Cai T, Pencina MJ, D'Agostino RB, Wei LJ. On the C-statistics for evaluating overall adequacy of risk prediction procedures with censored survival data. Stat Med 2011;30:1105-1117.
[27] Schemper M, Smith TL. A note on quantifying follow-up in studies of failure time. Control Clin Trials 1996;17:343-346.
[28] Duvoux C, Roudot-Thoraval F, Decaens T, Pessione F, Badran H, Piardi T, et al. Liver transplantation for hepatocellular carcinoma: a model including alpha-fetoprotein improves the performance of Milan criteria. Gastroenterology 2012;143:986-994 e983; quiz e914-985.
[29] Mazzaferro V, Sposito C, Zhou J, Pinna AD, De Carlis L, Fan J, et al. Metroticket 2.0 Model for Analysis of Competing Risks of Death After Liver Transplantation for Hepatocellular Carcinoma. Gastroenterology 2018;154:128-139.
[30] McMillan DC. The systemic inflammation-based Glasgow Prognostic Score: a decade of experience in patients with cancer. Cancer Treat Rev 2013;39:534-540.
[31] Hucke F, Pinter M, Graziadei I, Bota S, Vogel W, Muller C, et al. How to STATE suitability and START transarterial chemoembolization in patients with intermediate stage hepatocellular carcinoma. J Hepatol 2014;61:1287-1296.
[32] Mori S, Kita J, Kato M, Shimoda M, Kubota K. Usefulness of a new inflammation-based scoring system for prognostication of patients with hepatocellular carcinoma after hepatectomy. Am J Surg 2015;209:187-193.
[33] El-Khoueiry AB, Melero I, Yau TC, Crocenzi TS, Kudo M, Hsu C, et al. Impact of antitumor activity on survival outcomes, and nonconventional benefit, with nivolumab (NIVO) in patients with advanced hepatocellular carcinoma (aHCC): Subanalyses of CheckMate-040. J Clin Oncol 2018;36 (4_suppl):475-475.
[34] Finn RS, Qin S, Ikeda M, Galle PR, Ducreux M, Kim T-Y, et al. IMbrave150: Updated overall survival (OS) data from a global, randomized, open-label phase III study of

atezolizumab (atezo) + bevacizumab (bev) versus sorafenib (sor) in patients (pts) with unresectable hepatocellular carcinoma (HCC). Journal of Clinical Oncology 2021;39:267-267.
[35] Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell 2011;144:646-674.
[36] Diakos CI, Charles KA, McMillan DC, Clarke SJ. Cancer-related inflammation and treatment effectiveness. Lancet Oncol 2014;15:e493-503.
[37] Pinato DJ, Stebbing J, Ishizuka M, Khan SA, Wasan HS, North BV, et al. A novel and validated prognostic index in hepatocellular carcinoma: the inflammation based index (IBI). J Hepatol 2012;57:1013-1020.
[38] Sanghera C, Teh JJ, Pinato DJ. The systemic inflammatory response as a source of biomarkers and therapeutic targets in hepatocellular carcinoma. Liver Int 2019;39:2008-2023.
[39] Mantovani A, Allavena P, Sica A, Balkwill F. Cancer-related inflammation. Nature 2008;454:436-444.
[40] Yang J, Wezeman M, Zhang X, Lin P, Wang M, Qian J, et al. Human C-reactive protein binds activating Fcgamma receptors and protects myeloma tumor cells from apoptosis. Cancer Cell 2007;12:252-265.
[41] Naugler WE, Sakurai T, Kim S, Maeda S, Kim K, Elsharkawy AM, et al. Gender disparity in liver cancer due to sex differences in MyD88-dependent IL-6 production. Science 2007;317:121-124.
[42] Park EJ, Lee JH, Yu GY, He G, Ali SR, Holzer RG, et al. Dietary and genetic obesity promote liver inflammation and tumorigenesis by enhancing IL-6 and TNF expression. Cell 2010;140:197-208.
[43] Maeda S, Hikiba Y, Sakamoto K, Nakagawa H, Hirata Y, Hayakawa Y, et al. Ikappa B kinasebeta/nuclear factor-kappaB activation controls the development of liver metastasis by way of interleukin-6 expression. Hepatology 2009;50:1851-1860.
[44] Yoshida T, Ichikawa J, Giuroiu I, Laino AS, Hao Y, Krogsgaard M, et al. C reactive protein impairs adaptive immunity in immune cells of patients with melanoma. J Immunother Cancer 2020;8.
[45] Jimenez RV, Kuznetsova V, Connelly AN, Hel Z, Szalai AJ. C-Reactive Protein Promotes the Expansion of Myeloid Derived Cells With Suppressor Functions. Front Immunol 2019;10:2183.
[46] Akamine T, Takada K, Toyokawa G, Kinoshita F, Matsubara T, Kozuma Y, et al. Association of preoperative serum CRP with PD-L1 expression in 508 patients with non-small cell lung cancer: A comprehensive analysis of systemic inflammatory markers. Surg Oncol 2018;27:88-94.
[47] Morita M, Tamiya M, Fujimoto D, Tamiya A, Suzuki H, Hirano K, et al. Prediction of patients with a tumor proportion score > 50% who do not respond to first-line monotherapy with pembrolizumab. BMC Cancer 2020;20:93.
[48] Riedl JM, Barth DA, Brueckl WM, Zeitler G, Foris V, Mollnar S, et al. C-Reactive Protein (CRP) Levels in Immune Checkpoint Inhibitor Response and Progression in Advanced Non-Small Cell Lung Cancer: A Bi-Center Study. Cancers (Basel) 2020;12.
[49] Iivanainen S, Ahvonen J, Knuuttila A, Tiainen S, Koivunen JP. Elevated CRP levels indicate poor progression-free and overall survival on cancer patients treated with PD-1 inhibitors. ESMO Open 2019;4:e000531.
[50] Zhu AX, Kang YK, Yen CJ, Finn RS, Galle PR, Llovet JM, et al. Ramucirumab after sorafenib in patients with advanced hepatocellular carcinoma and increased alpha-

fetoprotein concentrations (REACH-2): a randomised, double-blind, placebo-controlled, phase 3 trial. Lancet Oncol 2019;20:282-296.
[51] Laderoute MP, Pilarski LM. The inhibition of apoptosis by alpha-fetoprotein (AFP) and the role of AFP receptors in anti-cellular senescence. Anticancer Res 1994;14:2429-2438.
[52] Terentiev AA, Moldogazieva NT. Alpha-fetoprotein: a renaissance. Tumour Biol 2013;34:2075-2091.
[53] Pardee AD, Shi J, Butterfield LH. Tumor-derived alpha-fetoprotein impairs the differentiation and T cell stimulatory activity of human dendritic cells. J Immunol 2014;193:5723-5732.
[54] Shan YF, Huang YL, Xie YK, Tan YH, Chen BC, Zhou MT, et al. Angiogenesis and clinicopathologic characteristics in different hepatocellular carcinoma subtypes defined by EpCAM and alpha-fetoprotein expression status. Med Oncol 2011;28:1012-1016.
[55] Meng W, Li X, Bai Z, Li Y, Yuan J, Liu T, et al. Silencing alpha-fetoprotein inhibits VEGF and MMP-2/9 production in human hepatocellular carcinoma cell. PLoS One 2014;9:e90660.
[56] Fukumura D, Kloepper J, Amoozgar Z, Duda DG, Jain RK. Enhancing cancer immunotherapy using antiangiogenics: opportunities and challenges. Nat Rev Clin Oncol 2018;15:325-340.
[57] Emerging Risk Factors C, Kaptoge S, Di Angelantonio E, Lowe G, Pepys MB, Thompson SG, et al. C-reactive protein concentration and risk of coronary heart disease, stroke, and mortality: an individual participant meta-analysis. Lancet 2010;375:132-140.

Tables

Table 1 Baseline characteristics of the training and validation cohort			
	Training set, n=190 (100%)	**Validation set, n=102 (100%)**	**Standardized differences**
Age (years), mean±SD	66.2±10.4	64.6±11.9	0.143
Sex			
Male	153 (81%)	83 (81%)	-0.022
Female	37 (19%)	19 (19%)	
Etiology			
Viral	55 (29%)	39 (38%)	-0.198
Non-viral	135 (71%)	63 (62%)	
Child-Pugh stage			
A	101 (53%)	72 (71%)	A vs. B/C -0.365
B	72 (38%)	28 (28%)	
C	17 (9%)	2 (2%)	
ECOG PS			
0	88 (46%)	46 (45%)	0.024
≥1	102 (54%)	56 (55%)	
Prior treatment	149 (78%)	82 (80%)	-0.049
Immunotherapy as systemic			
First-line	82 (43%)	35 (34%)	First-vs. second-/later-line 0.182
Second-line	57 (30%)	44 (43%)	
Later-line	51 (27%)	23 (23%)	
Macrovascular invasion	77 (41%)	33 (32%)	0.170
Extrahepatic metastasis	85 (45%)	55 (54%)	-0.184

BCLC stage			
A	2 (1%)	–	A/B vs. C/D 0.011
B	21 (11%)	12 (12%)	
C	149 (78%)	88 (86%)	
D	18 (10%)	2 (2%)	
Alpha-Fetoprotein			
<100 ng/ml	93 (49%)	56 (55%)	-0.119
≥100 ng/ml	97 (51%)	46 (45%)	
C-reactive protein			
<1 mg/dl	88 (46%)	43 (42%)	0.084
≥1 mg/dl	102 (54%)	59 (58%)	

Abbreviations: BCLC, Barcelona-Clinic Liver Cancer; ECOG PS, Eastern Cooperative Oncology Group Performance Status.

Table 2 Univariable and multivariable analyses of prognostic factors for overall survival in the training cohort

		Univariable		Multivariable	
		HR (95%CI)	**p-value**	**HR (95%CI)**	**p-value**
Etiology	Viral vs. non-viral	1.3 (0.8-2.0)	0.245		
Immunotherapy line	1^{st}-/2^{nd}-line vs. later-line	0.8 (0.5-1.3)	0.393		
Child-Pugh class	A vs. B/C	2.9 (1.9-4.2)	<0.001	2.3 (1.5-3.4)	<0.001
ECOG PS	0 vs. ≥1	2.6 (1.7-3.8)	<0.001	2.1 (1.4-3.2)	<0.001
Macrovascular invasion	Absent vs. present	1.3 (0.9-1.8)	0.241		
Extrahepatic metastases	Absent vs. present	0.9 (0.6-1.3)	0.532		
Alpha-Fetoprotein	<100 vs. ≥100 ng/ml	2.0 (1.3-2.9)	0.001	1.7 (1.2-2.6)	0.007
C-reactive protein	<1 vs. ≥1 mg/dl	2.1 (1.4-3.1)	<0.001	1.7 (1.2-2.6)	0.007

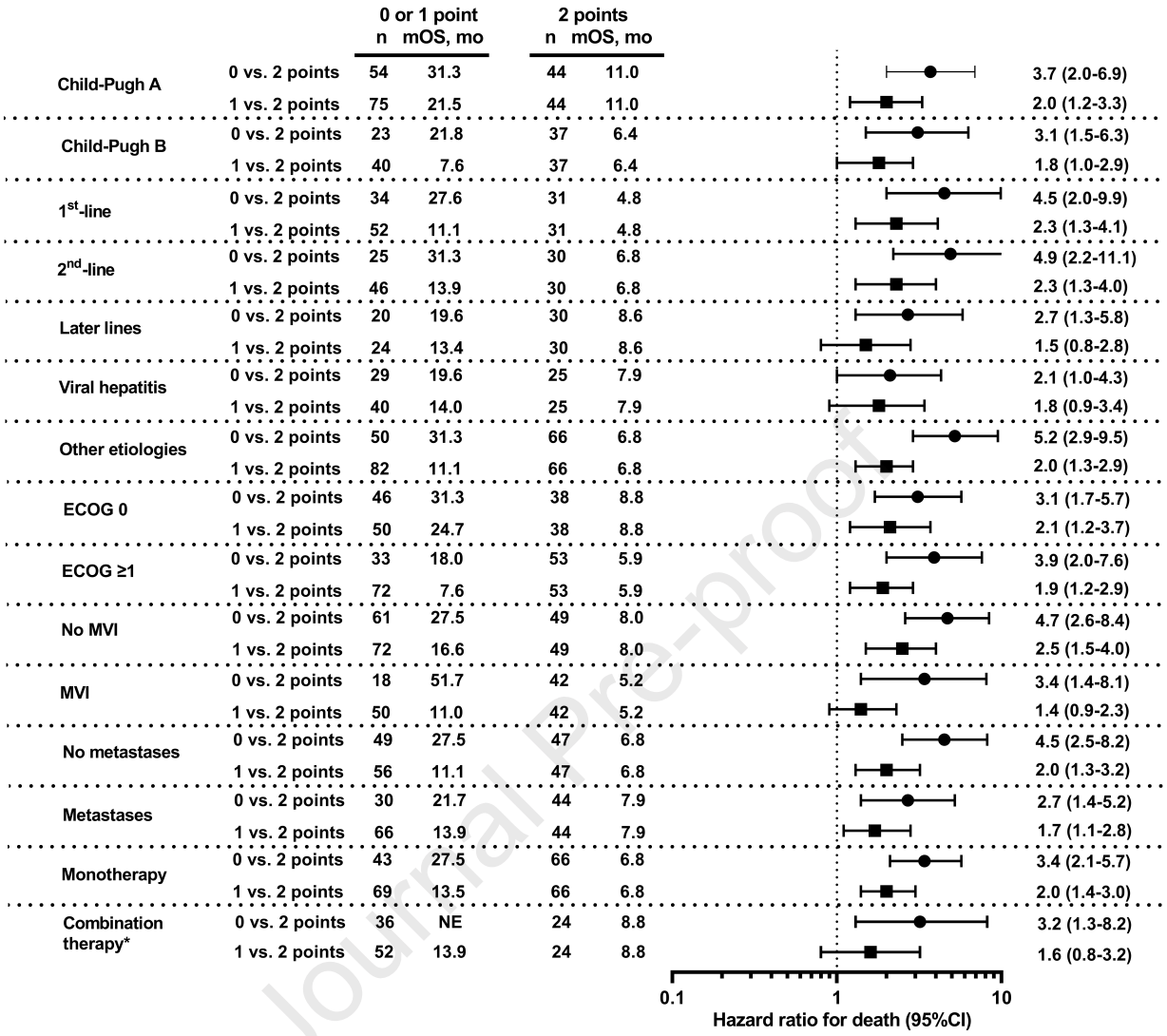
Abbreviations: ECOG PS, Eastern Cooperative Oncology Group Performance Status.

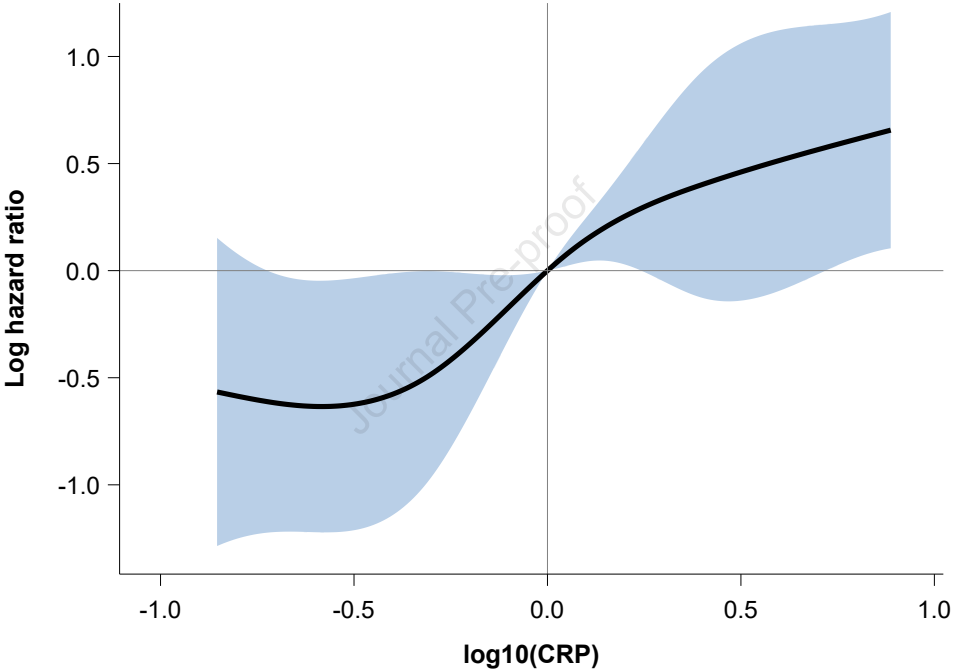
Table 3 Efficacy according to CRAFITY score in the training set

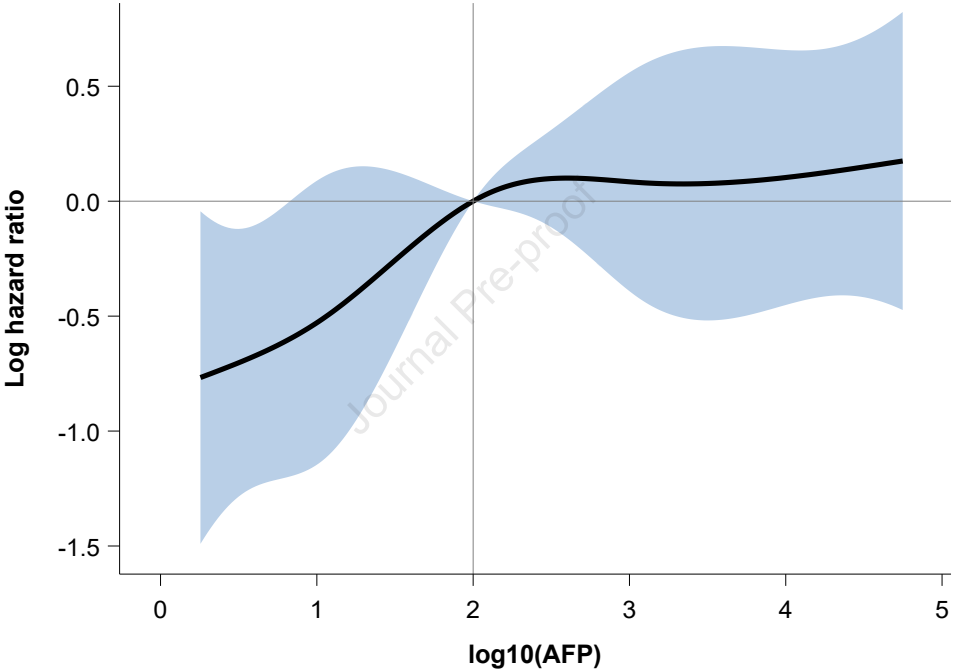
	CRAFITY low, n=53	**CRAFITY intermediate, n=75**	**CRAFITY high, n=62**
Overall survival			p<0.001
Median (95%CI), months	27.6 (19.5-35.8)	11.3 (8.0-14.6)	6.4 (4.8-8.1)
HR (95%CI)	1	2.0 (1.1-3.4)	3.6 (2.1-6.2)
Best radiological response*			p=0.001
Complete/partial response	13 (28%)	18 (28%)	8 (17%)
Stable disease	24 (52%)	23 (36%)	10 (22%)
Progressive disease	9 (20%)	23 (36%)	28 (61%)
Disease control*			p<0.001
Yes (CR/PR/SD)	37 (80%)	41 (64%)	18 (39%)
No (PD)	9 (20%)	23 (36%)	28 (61%)

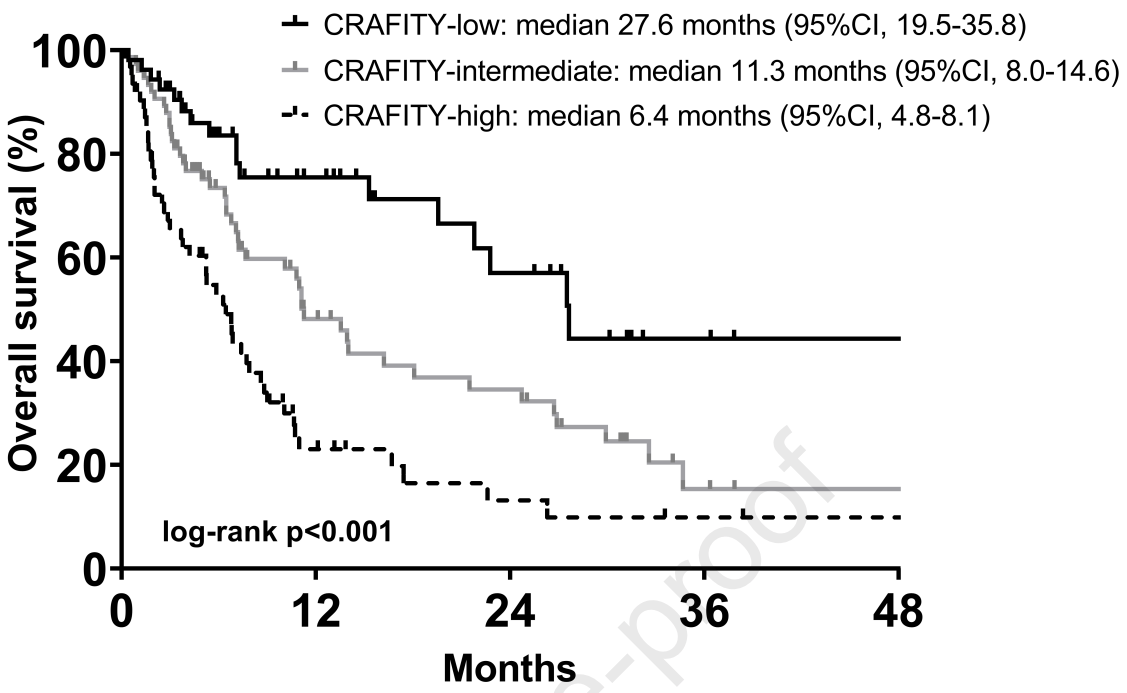
Abbreviations: CR, complete response; PD, progressive disease; PR, partial response; SD, stable disease.

* 156 of 190 (82%) patients had at least one follow-up imaging and were evaluable

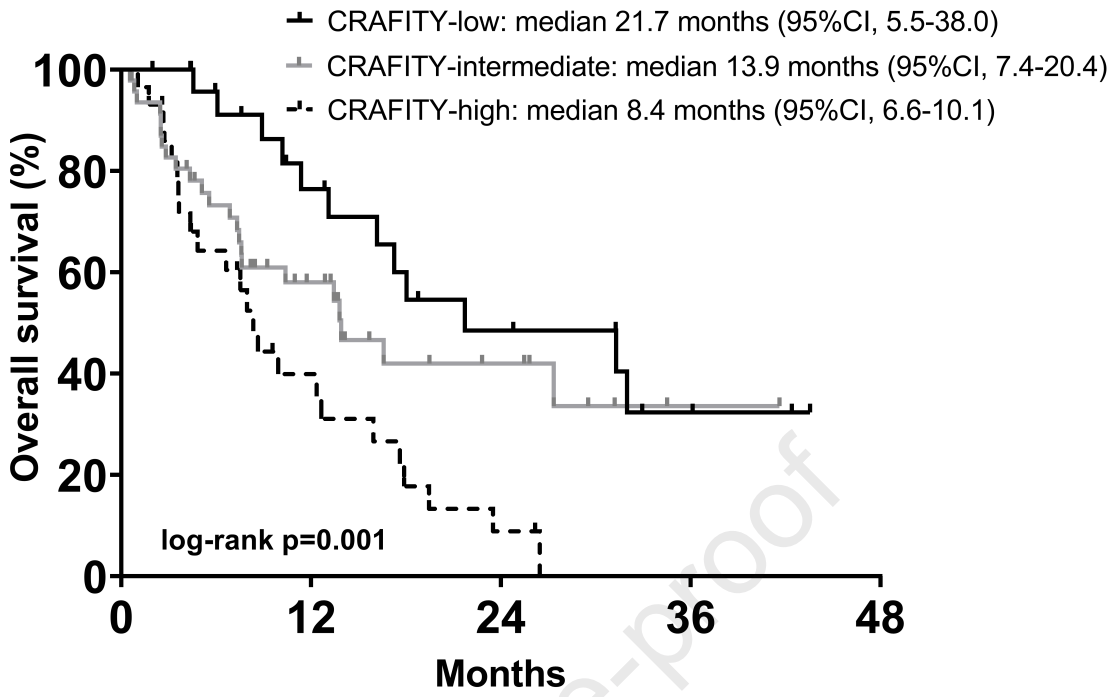




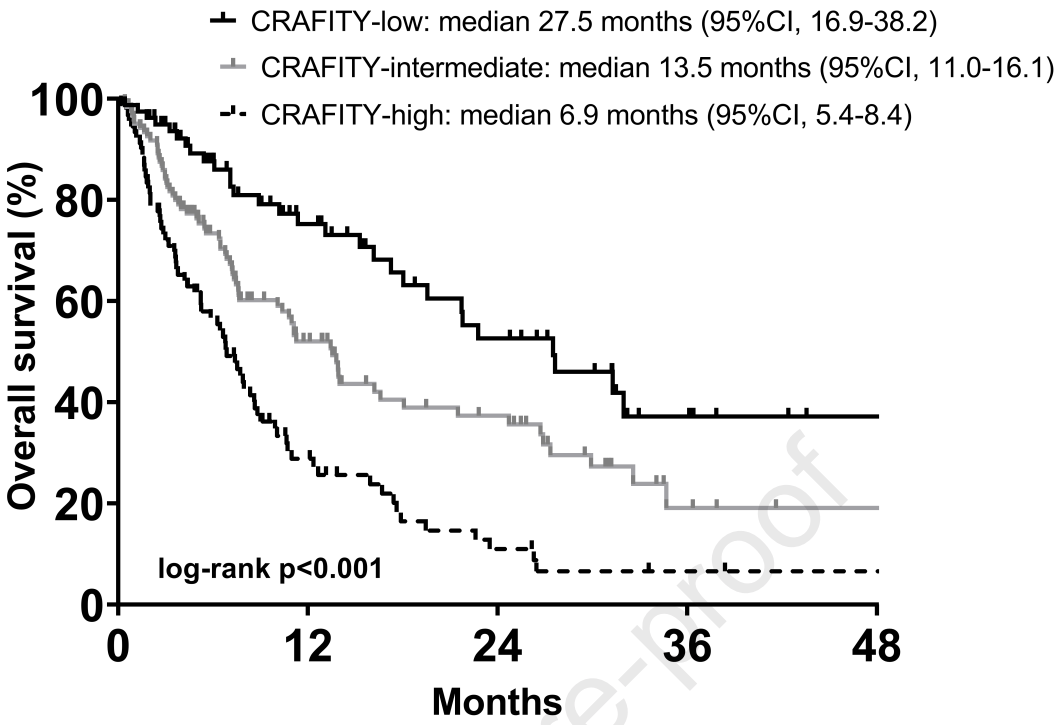




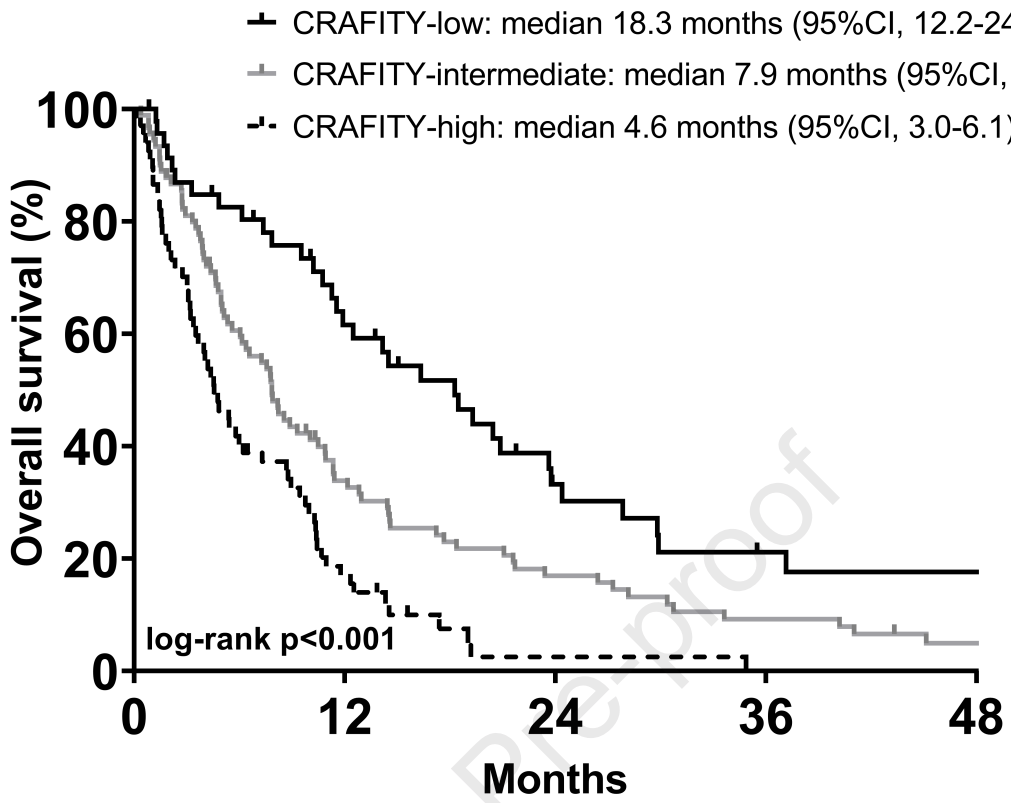
No. at risk					
0 points	53	22	12	3	1
1 point	75	24	15	3	1
2 points	62	10	4	2	1



No. at risk					
0 points	26	15	8	3	0
1 point	47	18	7	1	0
2 points	29	9	2	0	



No. at risk					
0 points	79	37	20	6	1
1 point	122	42	22	4	1
2 points	91	19	6	2	1



No. at risk					
0 points	47	26	11	6	5
1 point	90	28	14	7	3
2 points	67	11	1	0	

Figures

Figure 1. Patient flowchart.

Abbreviations: TACE, transarterial chemoembolization.

Figure 2. Restricted cubic spline analyses for CRP and AFP.

(A) Log hazard ratio function and 95 % pointwise confidence band estimated by a restricted cubic spline function for quantifying the effect of $\log_{10}$(CRP) on overall survival. Smaller log hazard ratios indicate better survival. A reference value of 1 mg/dl was used (that is a value of 0 on the decadic log-scale). The 5 spline knots where placed at -0.854, -0.310, 0.059, 0.403, and 0.879, which corresponds to the 5th, 27.5th, 50th, 72.5th and 95th percentile of the $\log_{10}$(CRP)-distribution, respectively.

(B) Log hazard ratio function and 95 % pointwise confidence band estimated by a restricted cubic spline function for quantifying the effect of $\log_{10}$(AFP) on overall survival. Smaller log hazard ratios indicate better survival. A reference value of 100 ng/ml was used (that is a value of 2 on the decadic log-scale). The 5 spline knots where placed at 0.255, 1.053, 2.102, 3.093, and 4.726, which corresponds to the 5th, 27.5th, 50th, 72.5th and 95th percentile of the $\log_{10}$(AFP)-distribution, respectively.

Abbreviations: CRP, C-reactive protein; AFP alpha-fetoprotein.

Figure 3. Kaplan-Meier survival curves according to CRAFITY score. Overall survival according to CRAFITY points in the training cohort (A), validation cohort (B), pooled cohort (C), and sorafenib cohort (D).

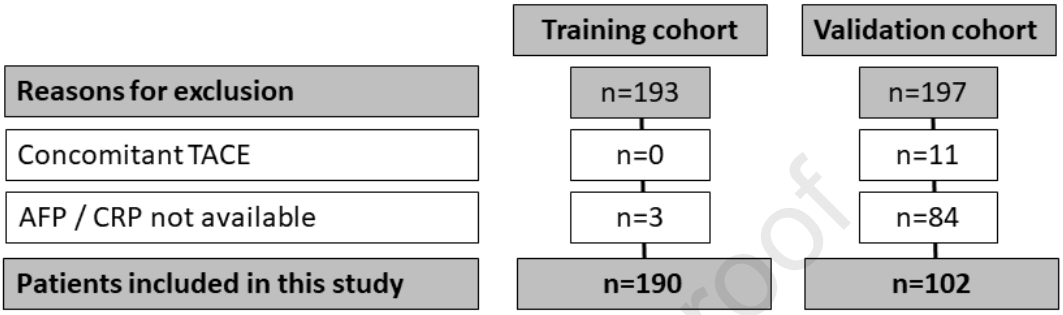
Figure 4. Median overall survival (OS) and hazard ratios for death comparing CRAFITY categories (0 vs. 2 and 1 vs. 2 points) in different subgroups in the pooled immunotherapy cohort.

* Including patients who received combination of PD-(L)1-targeted immunotherapy and VEGF-targeted agents.

Abbreviations: CI confidence interval; ECOG, Eastern Cooperative Oncology Group Performance Status; mOS, median overall survival; MVI, macrovascular invasion; NE, not evaluable.

Centers (in alphabetical order):

1. **Training cohort: Frankfurt (DE), Hamburg (DE), Hanover (DE), Klagenfurt (AT), Mainz (DE), Vienna (AT)**
2. **Validation cohort: Bellinzona (CH), Bern (CH), Cologne (DE), Esslingen (DE) Mannheim (DE), Milan (IT), Tuebingen (DE), Zurich (CH)**



Highlights:

- Baseline serum alpha-fetoprotein ≥100 ng/ml and C-reactive protein ≥1 mg/dl were independently associated with worse overall survival in patients with hepatocellular carcinoma (HCC) treated with immune checkpoint blockers (ICB)
- A score based on these two variables predicts disease control rate and overall survival in HCC patients treated with ICB-based systemic therapies
- The score was validated in an independent cohort of patients with HCC who received ICB-based systemic therapies
- In HCC patients treated with sorafenib, the score was prognostic for overall survival but not predictive for disease control rate