IDENTIFYING STAGE 1 HEPATOCELLULAR CARCINOMA PATIENTS WITH POOR PROGNOSIS

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ABSTRACT. **Aim:** The purpose of this note is to show that survival times for stage 1 HCC patients has a bimodal distribution, and to present a genetic signature for identifying the high-risk group.

**Methods:** Clinical data from TCGA gives the survival distribution. A predictive signature is extracted from TCGA gene expression data using the LUST algorithm.

**Results:** About 20% of stage 1 liver cancer patients die within the first two years, while 70% live at least four years, often much longer. A genetic signature of 44 genes related to immune response identifies the high-risk group.

**Conclusion:** Since the patients in the high-risk group can be recognized with fair accuracy, they should be potential candidates for adjuvant therapy.

**Introduction.** Early diagnosis of hepatocellular carcinoma (HCC) is perhaps the primary factor for patient outcome with liver cancer [1]. Patients treated for stage 1 HCC have a good chance of surviving five years or longer. However, the survival distribution for stage 1 HCC patients is bimodal: roughly 20% of these patients die within the first two years after diagnosis, another 10% during the next two years, while 70% live at least four years, often much longer. This is illustrated with a histogram in Figure 1, and with the standard empirical risk curve for censored data in Figure 2. The survival histogram projected from the ecdf curve is given in Figure 3.

If the poor prognosis group could be recognized, then these patients would be candidates for adjuvant treatment. While the patients in the short-term survival group have other liver disease, such as hepatitis or cirrhosis, they are not different in that respect from the long-term survivors for whom treatment is effective. Moreover, the gene expression

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Figure 1. Survival histogram for stage 1 liver cancer patients, based on TCGA clinical records for 157 stage 1 HCC patients with tumors weighing under 500 grams. The mean survival time, censored and uncensored, for this group was 910 days. This histogram represents those 83 patients who either (1) died at any time, or (2) survived at least 910 days. For patients in the second group who are still alive, the number of days to last follow-up is used in place of days to death. Thus it greatly underestimates survival times for the low-risk group, as only 10 of the 54 patients indicated at times > 910 days were actually deceased.

Profile for stage 1 HCC patients is very different from that for later stages, which suggests that we look there for markers.

Methods. Clinical and mRNA expression data for HCC patients was obtained from The Cancer Genome Atlas (TCGA). The data for stage 1 patients with tumor weight less than 500 grams was extracted, giving 157 subjects. (Later analysis showed that excluding the larger tumors had little effect on the results.) All calculations were done in MATLAB (MathWorks, Natick, MA, USA).
Figure 2. Risk curve (probability of death) for stage 1 liver cancer patients. Note how the curve flattens between 700 and 1400 days.

The mRNA expression data was analyzed using the LUST algorithm to find sets of genes with coordinated expression patterns [2]. This is a two-step algorithm. The first step looks for sets of genes that maximize a graph-theoretic objective function, unsupervised by clinical information. These metagenes are then refined in the second step, using clinical data to find subsets that separate the Kaplan-Meier survival curves. The algorithm produces a number of signatures predicting survival, and ranks them.

For the stage 1 liver patients, a signature consisting of 44 genes relating to immune response was chosen by the algorithm. (These genes are listed at the end of the text.) Each patient is assigned a score which is a linear combination of the expression levels for these genes, as in [2] or [3]. Patients with a score higher than a chosen threshold were assigned to the high-risk group. The results were fairly constant over a range of thresholds, as indicated by the ROC curve in Figure 4.
Results. Let us designate patients who die in the first 750 days after diagnosis as short-term survivors. To analyze the performance of the signature for predicting short-term survivors, subjects who are censored at less than 750 days are removed from the data set. If we use a test score threshold of $s = .0005$ (in the middle of the first plateau on the ROC curve), the test correctly places about 67% (18/27) of the short-term survivors in the high-risk group (true positive rate). The true negative rate with this threshold is 85% (51/60). This analysis is illustrated in Figure 5.

The survival statistics are based on deaths from all causes. Of the 27 deaths during the first two years, 11 occurred during the first four months ($\leq 120$ days). These could perhaps be attributed to general liver disease or complications of surgery; the clinical record does not specify. The test places 7 of these 11 in the high-risk group.
The remaining 16 deaths under two years occurred between 170 and 700 days. Of these, 7 were due to recurrence of liver cancer, 5 were due to extrahepatic recurrence, while the cause for 4 deaths was not specified in the record. The test puts 11 of these 16 patients in the high-risk group.

Some other risk factors for liver cancer failed to predict the short-term survivors. Fibrosis and hepatitis B even appeared to convey a slight patient benefit, though this is surely a statistical anomaly. Most of the patients with larger tumors were censored, so no conclusion could be drawn there. However, 8 of the 28 short-term survivors (29%) had hepatitis C, as compared with 24 of the remaining 127 stage 1 HCC patients (19%).

Running the LUST algorithm using disease-free survival, instead of survival for the second step, produces a related 4-gene signature that predicts recurrence with some accuracy: CD53, EVI2B, IKZF1, LCP2. (The 44-gene signature does not do this because patients in the low-risk group disproportionately tend to survive recurrence.) If we remove the
Figure 5. This graph plots each patient’s score on the 44-gene test vs. survival. The score $s$ on the test, arranged in increasing order, is indicated by the blue curve, in units of .001 on the left $y$-axis. The vertical green line represents a cutoff at $s = .0005$, so that patients to the right of the green line are in the high-risk group, and patients to the left represent the low-risk group. Survival is measured on the right $y$-axis in days. The horizontal green line is at 750 days (just over 2 years). Deaths are indicated by a red +, while survival times for patients with censored survival times over 750 days are indicated by a blue ◦. Censored patients with survival times less than 750 days are omitted. Thus the lower right hand corner represents true positives, the upper right hand quadrant is false positives, the upper left hand quadrant is true negatives, and the lower left hand corner is false negatives.

patients who died before 120 days and set an appropriate threshold, then 62% (21/34) patients in the high-risk group had recurrence, with
15 of liver cancer and 6 extrahepatic tumors. The low-risk group had 30% recurrence (34/113), including 4 distant recurrences.

**Discussion.** For the majority of patients diagnosed with early stage HCC and small tumors, treatment involving primarily resection is effective [4]. However, there is a distinct cohort, consisting of about 20% of these patients, that is at great risk during the first two years after diagnosis. The main objective of this note is to distinguish this cohort as a group that needs to be considered separately as potential candidates for alternate therapy.

The second point is that high-risk stage 1 HCC patients can be identified using a genetic signature. This suggests genetic testing after resection or transplantation as part of a rubric, along with clinical considerations, to identify high-risk patients. Refinements of the current 44-gene signature may well be possible.

All this suggests that the high-risk group could benefit from additional treatment, but necessarily begs the question of what form that alternate treatment might take. That is a more difficult clinical question, not to be answered from a broad data analysis. The options for adjuvant treatment of HCC are very limited, and many patients would not be candidates for further treatments because of poor liver function. The fact that the signature is comprised of immune regulatory genes suggests that perhaps some sort of immunotherapy could be appropriate; see e.g. [5, 6, 7, 8]. The data could also be interpreted as supporting increased use of neoadjuvant therapy for early stage liver cancer [9, 10].

The increasing incidence of HCC makes finding an effective treatment for the high-risk group a problem worthy of attention.

**The 44-gene signature.** ACAP1, ARHGAP9, BIN2, C1QB, C1QC, CCR5, CD2, CD3D, CD3E, CD48, CD52, CD53, CD5, CD6, CORO1A, COTL1, DOCK2, EVI2A, HCLS1, IKZF1, IL2RG, ITGB2, LAPTM5, LCK, LSP1, LY9, MAP4K1, MYO1G, NCKAP1L, PIK3CD, PLEK, PSTPIP1, PTPN7, RAC2, RLTPR, RUNX3, SASH3, SELPLG, SIRPG, SIT1, SLAMF8, SLA, TMC8, ZAP70.

1. **Declarations**

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