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### PERITUMORAL TISSUE IN PATIENTS DIAGNOSED WITH HEPATOCELLULAR CANCER AFTER HCV CURE SHOWS STUBBORN B CELLS AND MACROPHAGE ENRICHMENT

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**Background:** New hepatitis C virus (HCV) direct-acting antiviral drugs achieve high sustained viral response (SVR) rates in cirrhotic patients. Despite clinical HCV cure, liver injury and inflammation, including B cell abnormalities, persist in 25-66% of these patients and HCC risk remains elevated. It is important to define the mechanisms of persistent inflammation and to determine their relationship to tumorigenesis after HCV cure. CXCL13 (also called B cell-attracting chemokine 1) is involved in hepatic B-lymphocyte trafficking and lymphoid follicle development. **Methods:** Liver samples were obtained from 29 HCC patients who underwent liver transplant in Rush University Medical Center from 2015 to 2017. Three groups were defined: HCV antibody (AB)<sup>+</sup>/HCV RNA<sup>-</sup> (N=7, cured), HCV AB<sup>+</sup>/HCV RNA<sup>+</sup> (N=11, chronic) and HCV AB<sup>-</sup> (N=11, controls) (Table 1). Immunostaining of non-tumor areas of the liver delineated the inflammatory infiltrate (anti-CD3, CD19, CD38, CD68); anti-CXCL13 measured B cell recruitment and anti-MDA5 indicated responses to interferon- $\alpha$  stimulation. Staining was objectively quantified by integrated density with Image J. The Tabula Muris database, which was used to identify liver cells that express CXCL13, flagged the macrophage population. **Results:** The most striking result was the high expression of CXCL13 in patients before and after HCV cure. Expression was similar in patients with chronic infection and in patients cured of HCV; the level in both was significantly higher than in controls,  $p \leq 0.05$ . Expression of CD19 (B cell marker), CD68 (macrophage/Kupffer cell marker) and MDA5 were significantly increased in both groups of HCV-exposed patients compared to controls. There was a statistically significant correlation ( $r=0.40$ ,  $p=0.03$ ) between CD68 staining and CXCL13 staining, indicating that hepatic macrophages contribute to B cell recruitment before and after HCV cure. The two groups of HCV-exposed patients and the controls had similar staining of CD3 (T and NKT cells) and CD38 (a marker of plasma cells). **Conclusion:** Patients who develop HCC in the first year after HCV cure have immunohistological evidence of persistent Interferon- $\alpha$  stimulated gene activation and enriched B cell inflammation potentially the consequence of CXCL13 production by resident long lived self-renewable hepatic macrophages.

Table Characteristic of patient information

	Male (%)	Age (Years) (Average $\pm$ SD)	HCV RNA (IU/ml) (Average)	Time from HCV RNA undetectable to liver transplant (Average $\pm$ SD)	MELD at LT (Ave $\pm$ SD)	MELD-Na at LT (Ave $\pm$ SD)
HCVAB(+)/HCVRNA(+) (N=11)	6 (54.5)	61.6 $\pm$ 3.7	3.83E+06	NA	18.0 $\pm$ 10.1	19.2 $\pm$ 10.2
HCVAB(+)/HCVRNA(-) (N=7)	6 (85.7)	63.1 $\pm$ 2.0	Undetectable	12 $\pm$ 6.7	12.5 $\pm$ 2.9	14.3 $\pm$ 2.6
HCVAB(-)/HCVRNA(-) (N=11)	8 (72.7)	64.4 $\pm$ 4.2	NA	NA	18.0 $\pm$ 10.7	19.0 $\pm$ 11.5

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### DECLINE OF PREVALENCE OF RESISTANCE ASSOCIATED SUBSTITUTIONS TO NS3 AND NS5A INHIBITORS AT DAA-FAILURE IN HEPATITIS C VIRUS IN ITALY OVER THE YEARS 2015 TO 2018

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**Background:** A minority of patients fails to eliminate HCV and resistance-associated substitutions (RASs) are commonly detected at failure of interferon-free DAA regimens. **Methods:** Within the Italian network VIRONET-C, the prevalence of NS3/NS5A/NS5B RASs was retrospectively evaluated in patients who failed an EASL recommended DAA-regimen in 2015-2018. The geno2pheno system and Sorbo MC et al. Drug Resistance Updates 2018 were used to infer HCV-genotype/subtype and predict drug resistance. The changes in prevalence of RASs over time were evaluated by chi-square test for trend, predictors of RASs at failure were analysed by logistic regression. **Results:** We included 386 HCV infected patients: 75% males, median age was 56 years (IQR 52-61), metavir fibrosis stage F4 in 76%; 106 (28%) were treatment-experienced: 91 (86%) with IFN-based treatments, 26 (25%) with DAAs. Patients with HIV and HBV coinfection were 10% (33/317) and 8% (6/72), respectively. HCV genotype was 1b in 122 pts (32%), 3 in 109 (28%), 1a in 97 (25%), 4 in 37 (10%), 2 in 21 (5%). DAA regimens were: LDV/SOF in 115 (30%), DCV/SOF in 103 (27%), 3D in 83 (21%), EBR/GRZ

in 32 (8%), VEL/SOF in 29 (7%), GLE/PIB in 18 (5%) and 2D in 6 (2%); ribavirin was administered in 123 (32%). The NS5A fasta-sequence was available for all patients, NS5B for 361 (94%), NS3 for 365 (95%). According to the DAA failed the prevalence of any RASs was 90%, namely 80/135 (59%) in NS3, 313/359 (87%) in NS5A, 114/286 (40%) in NS5B. The prevalence of any RASs significantly declined from 2015 to 2018 (93% vs 70%,  $p=0.004$ ): NS5A RASs from 90% to 72% ( $p=0.29$ ), NS3 RASs from 74% to 18% ( $p<0.001$ ), while NS5B RASs remained stable. Independent predictors of any RASs included advanced fibrosis (AOR 6.1, CI 95% 1.8-20.3,  $p=0.004$ ) and genotype (G2 vs G1a AOR 0.03, CI 95% 0.002-0.31,  $p=0.004$ ; G3 vs G1a AOR 0.08, CI 95% 0.01-0.62,  $p=0.02$ ; G4 vs G1a AOR 0.05, CI 95% 0.006-0.46,  $p=0.008$ ), after adjusting for age, previous HCV treatment and year of genotype. Notably, full activity was predicted for GLE/PIB in 75% of cases and for at least two components of VEL/SOF/VOX in 53% of cases, no case with full-resistance to either regimen was found. **Conclusion:** Despite decreasing prevalence over the years, RASs remain common at virological failure of DAA treatment, particularly in patients with the highest grade of liver fibrosis. The identification of RASs after failure could play a crucial role in optimizing retreatment strategies.

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