The *Liver Cancer in HIV Study Group*

A global multi-center retrospective cohort study
to investigate hepatocellular carcinoma in HIV-infected patients

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SUMMARY:

The *Liver Cancer in HIV Study Group* collects cases of HIV-infected patients with proven hepatocellular carcinoma (by AASLD 2005 diagnostic criteria) starting in 1992 from sites around the world. Each site investigator will collect cases already known to her/his center and also investigate for other (previously unknown) cases through the local Tumor /Cancer Registry, wherever feasible. Data collection of each subject is performed retrospectively. A variety of demographic, clinical, imaging and laboratory data will be collected as outlined in a specific case report form (CRF) that contains explanations on data collection.

Many data analyses from this database can be performed. They will be driven by investigators in this study group. Publications will try to include as many investigators as possible in the author list, and individual contributions will be considered.

BACKGROUND:

Since the introduction of highly-active antiretroviral therapies, patients with HIV infection lead longer and healthier lives. However, they are now subject to comorbidity and mortality from illnesses not directly related to HIV, chief among them chronic liver disease from hepatitis B or C virus (HCV or HBV) coinfection. Many studies have been published on the topics of epidemiology, natural history, and therapy of HIV/HCV and also of HIV/HBV coinfections.

However, the problem of hepatocellular carcinoma (HCC) in HIV-infected patients has only recently surfaced,[1] and the literature on this topic has been sparse. As of March 2010, only 3 case series of HCC in HIV-infected individuals have been published on HCC in HIV-positive patients. A case series of 7 HIV/HCV-coinfected subjects with HCC from Madrid,[2] a study of 41 HIV-positive patients (any etiology) from Italy,[3] and a study of 63 subjects from this study group when it was still confined to North America only.[4]

Since the expansion in 2009 to multiple sites outside of North America, one poster on HCC screening in HIV/HCV-coinfected patients was presented at the 17th Conference on Retroviruses and Opportunistic Infections (CROI 2010) in San Francisco in February 2010.[5] Another two posters have been accepted for the 45th annual meeting of the European Association for the Study of the Liver (EASL 2010) in Vienna in April 2010.

AIM:

The aim of this study group is to shed further light on the issue of HCC in HIV-infected subjects with focus on endpoints like initial presentation, therapies, and outcome. This will be accomplished by accumulating a large database with at least N=250 subjects to have enough statistical power to show small differences at a $p<0.05$ level and perform multi-variable

regression analyses with a high number of endpoints so that many variables can be tested for independent correlation.

As of the date of this protocol, 12 March 2010, a total of N=128 subjects have been contributed from 24 sites in 6 countries on 3 continents. The goal is to expand the study group to include all regions of the world to maximize representation of patients from various ethnic and geographic backgrounds. Publications in peer-reviewed journals and abstract presentations at international meetings may help expand the network.

PATIENT POPULATION

Each investigator will first collect known cases of HCC in HIV-infected patients at her/his center starting in 1992 (first anti-HCV testing). In addition, a systematic search of the local tumor/cancer registry for cases of HCC from 1992 forward should be made, and cases who are HIV-positive included in addition to known cases. Older cases may have been unrecognized or maybe unknown to current clinicians.

In order to be included in the database, a subject has to meet all three entry criteria

(1) HIV-seropositive

(2) Test result of anti-HCV available (anti-HCV testing started 1992)

(3) Confirmed HCC diagnosis by AASLD 2005 criteria

Only HIV-seropositive patients are being studied under this protocol. Their anti-HCV test result needs to be known, since chronic hepatitis C is the most common cause of liver disease and HCC in the Western world. Where possible, patients should also have results of HBV testing available, at least hepatitis B surface antigen, HBsAg and antibodies to hepatitis B core and surface antigens, anti-HBc and anti-HBs. In patients who are HBsAg-positive, results of HBeAg, anti-HBe, and HBV DNA level (note IU/mL or copies/ml) should be collected as well.

Diagnostic criteria according to the AASLD 2005 guidelines (Bruix J & Sherman M, *Hepatology*, Nov-2005) [6] must be met. They are

(1) Cytology or histology

(2) Imaging for mass >2.0 cm:
- (a) Hypervascular mass (early arterial phase contrast enhancement) on CT or MRI + AFP ≥200 ng/ml,
- (b) Hypervascular mass on imaging x 2 (CT and MRI) with any AFP,
- (c) Hypervascular mass AND early venous wash-out in one study (CT or MRI) with any AFP.

(3) Imaging for masses 1.0 – 2.0 cm:
hypervascular mass and early venous washout in one study (CT or MRI) with any AFP
This is new in the upcoming AASLD 2010 criteria (Bruix J & Sherman M, Hepatology,2010, in press, personal communication Morris Sherman), previously 2 studies were required.

Exemptions for the anti-HCV rule may be granted in rare cases, e.g. in resource-limited countries with very low prevalence of HCV infection, where HCV testing is not done routinely.

DATA COLLECTED:

All collected data are included in the standardized case report form (CRF) for each center. The CRF also has a box with explanations on data collection, as outlined below.

Demographics

- Age (via date of birth)
- Sex
- Race/ethnicity

Etiology of HCC (HCV, HBV, alcohol, etc.)

Date and type of diagnosis

- Date when the patient first meets AASLD 2005 criteria by either tissue diagnosis or imaging.
- How was diagnosis made (tissue, imaging, AFP)?

Date of death (or date last seen alive).

Initial presentation by symptoms (if yes, which ones) or by abnormal screening tests (imaging, AFP)

Imaging results closest to diagnosis

- Number of liver mass lesions
- Size (diameter) of largest mass.
- Is mass infiltrating?
- Evidence of splenomegaly?
- Evidence of cirrhosis?

Other tumor characteristics

- Presence of extra hepatic metastases
- Presence of portal vein (PV) thrombosis

HCC Therapy, all modalities ever given

- Potentially curative therapy, such as radiofrequency ablation [RFA], ethanol injection, surgical resection, or orthotopic liver transplantation [OLT], including the start date of the first such therapy. Type and start of 1st potentially curative therapy

- Effective, non-curative therapy, like transarterial chemoembolization [TACE] or sorafenib, including the start date of the first such therapy. Type and start of 1st non-curative effective therapy
- Future treatment modalities, like novel systemic chemotherapies, radiation, or others will be included only if they are proven to be effective (improve overall survival) in adequately-sized randomized controlled trials. If such prove does not exist at the time of data analysis, they will fall under the category "no or ineffective therapy".

HCC Therapy, subanalysis

- In patients treated with sorafenib (or future effective, but non-curative treatment modalities), data will also be collected separately on treatment duration and dose, incidence of side effects, effect on survival and tumor size, and other treatment-relevant information.

Antiviral therapy

- HIV therapy: start of first antiretroviral (ARV) therapy, medications of current ARV therapy
- HCV therapy: first treatment – start and end date, medications, viral response (sustained viral response [SVR], response-relapse [resp-rel], viral non-response [NR]). Same information for 2nd course of treatment if applicable. Information will only be collected on the 2 longest treatments, inlcuding the curative one, if applicable.
- HBV therapy: first treatment – start and end date, medications, viral response (lowest HBV DNA level on therapy, length of undetectable HBV DNA). Same information for 2nd course of treatment if applicable. Information will only be collected on the 2 longest treatments

Prior HCC screening activity:

- Date of last mass-free imaging study prior to 1st suspicion of HCC and type of imaging
- Date and value of last AFP measurement prior to 1st suspicion of HCC and type of imaging

Laboratory values closest to the date of diagnosis:

- Alfa-fetoprotein (AFP, including local upper limit of normal, ULN)
- Liver-related chemistry: AST, ALT (incl. local ULN), gamma-GT, total bilirubin, albumin
- Coagulation: prothrombin time, international normalized ratio (INR)
- Others: platelet count, iron, ferritin, total iron-binding capacity (TIBC)
- HIV-related labs: plasma HIV RNA, CD4+ cells (absolute and %)
- HCV-related labs: plasma HCV RNA, HCV genotype
- HBV-related labs: HBsAg, anti-HBc, anti-HBs, and where HBsAg is positive, also HBeAg, anti-HBe, plasma HBV DNA level

Other Clinical data:

- Child-Turcotte-Pugh (CTP) scores for ascites and hepatic encephalopathy
- HCV risk factor(s), date of onset of risk factor (= presumed date of infection)
- Performance Status Test (PST) as per Barcelona Clínic Liver Cancer criteria[7]
- Alcohol consumption (none-moderate-excessive)
- More clinical data may be added as the study progresses.

ANALYSES

Any investigator of the *Liver Cancer in HCC Study Group* is free to make suggestions for analysis of the database, and all suggestions will be explored for feasibility.

Currently suggested analyses include

(1) Effect of HCC screening on outcome in HIV/HCV-coinfected patients
(2) Effect of plasma HIV RNA level on outcome
(3) Frequency of HCC in HIV-infected patients over time
(4) Continental/regional differences of HCC in HIV-positive patients
(5) Differences between HIV/HCV- and HIV/HBV-coinfected patients with HCC
(6) Serum markers for cirrhosis at time of HCC diagnosis
(7) Comparison of published HCC staging systems in predictability of survival in HIV-infected patients
(8) Subanalysis of HIV-infected patients with HCC who received sorafenib.

Statistical analysis

Continuous variables are compared by Student's t-test (for normally distributed values)[8] or the Mann-Whitney U test[9] (not normally distributed), and categorical variables are compared using Chi-square analysis or Fisher's exact test[10] as appropriate. Survival between groups is compared using Kaplan-Meier analysis[11] and log rank testing, and correlation between patient variables and survival is analyzed through Cox proportional hazard analysis.[12] Multi-variable analysis is applied for all factors correlated with survival at $p<0.10$ in univariate Cox analysis. Correlation between patient variables and categorical outcome variables are made by univariate and multi-variable logistic regression analysis and by linear regression analysis for continuous outcome variables. Other statistical tests may be added as indicated.

PUBLICATIONS

By contributing cases to the study group database, each investigator is encouraged to submit ideas for analysis of the entire database.

As a general rule, the study group will attempt to give authorship to as many investigators as is feasible. Authorship in abstract presentations at international meetings and peer-review publications will be determined by individual contributions of each investigator, including among other factors number of cases added and recruitment of new sites and investigators. Publication committees may be established *ad hoc* to resolve any potential conflicts.

REFERENCES

1. Bruno R, Sacchi P, Filice C, Puoti M, Filice G. Hepatocellular carcinoma in HIV-infected patients with chronic hepatitis: an emerging issue. J Acquir Immune Defic Syndr 2002; 30(5):535-536.

2. Garcia-Samaniego J, Rodriguez M, Berenguer J, Rodriguez-Rosado R, Carbo J, Asensi V et al. Hepatocellular carcinoma in HIV-infected patients with chronic hepatitis C. Am J Gastroenterol 2001; 96(1):179-183.

3. Puoti M, Bruno R, Soriano V, Donato F, Gaeta GB, Quinzan GP et al. Hepatocellular carcinoma in HIV-infected patients: epidemiological features, clinical presentation and outcome. AIDS 2004; 18(17):2285-2293.

4. Bräu N, Fox RK, Xiao P, Marks K, Naqvi Z, Taylor LE et al. Presentation and outcome of hepatocellular carcinoma in HIV-infected patients: a U.S.-Canadian multicenter study. J Hepatol 2007; 47(4):527-537.

5. Núñez M, Kikuchi L, Barreiro P, Nelson M, Vispo ME, Page E et al. Screening for Hepatocellular Carcinoma (HCC) in HIV/HCV-Coinfected Patients: Impact on Staging, Therapy, and Survival. Presented at the 17th Conference on Retroviruses and Opportunistic Infections, San Francisco, CA, 16-19 February 2010, Abstract No 685 2010.

6. Bruix J, Sherman M. Management of hepatocellular carcinoma. Hepatology 2005; 42(5):1208-1236.

7. Llovet JM, Bru C, Bruix J. Prognosis of hepatocellular carcinoma: the BCLC staging classification. Semin Liver Dis 1999; 19(3):329-338.

8. Pearson ES. The probable error of a mean. Biometrika 1908; 6(1):1-25.

9. Mann HB, Whitney DR. On a test of whether one of 2 random variables is stochastically larger than the other. Annals of Mathematical Statistics 1947; 18:50-60.

10. Fisher RA. On the interpretation of Chi-square from contingency tables, and the calculation of P. Journal of the Royal Statistical Society 1922; 85(1):87-94.

11. Kaplan EL, Meier P. Nonparametric estimation from incomplete observations. Journal of the American Statistical Association 1958; 53:457-481.

12. Cox DR. Regression models and life tables. Journal of the Royal Statistical Society Series B 1972; 34:187-220.