Research Article

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Tremelimumab in combination with ablation in patients with advanced hepatocellular carcinoma

Austin G. Duffy[1], Susanna V. Ulahannan[1], Oxana Makorova-Rusher[1], Osama Rahma[1], Heiner Wedemeyer[2], Drew Pratt[3], Jeremy L. Davis[4], Marybeth S. Hughes[4], Theo Heller[5], Mei ElGindi[1], Ashish Uppala[1], Firouzeh Korangy[1], David E. Kleiner[3], William D. Figg[6], David Venzon[7], Seth M. Steinberg[7], Aradhana M. Venkatesan[8], Venkatesh Krishnasamy[8], Nadine Abi-Jaoudeh[8], Elliot Levy[8], Brad J. Wood[8], Tim F. Greten[1,*]

[1]*Gastrointestinal Malignancies Section, Thoracic and GI Oncology Branch, Center for Cancer Research, National Cancer Institute, National Institutes of Health, USA;* [2]*Department of Gastroenterology, Hepatology and Endocrinology, Hannover Medical School, Germany;* [3]*Laboratory of Pathology, Center for Cancer Research, National Cancer Institute, National Institutes of Health, USA;* [4]*Thoracic and GI Oncology Branch, Center for Cancer Research, National Cancer Institute, National Institutes of Health, USA;* [5]*Translational Hepatology Unit, Liver Diseases Branch (LDB), National Institute of Diabetes and Digestive and Kidney Disease (NIDDK) National Institutes of Health, USA;* [6]*Clinical Pharmacology Program, Center for Cancer Research, National Cancer Institute, National Institutes of Health, USA;* [7]*Biostatistics and Data Management Section, Center for Cancer Research, National Cancer Institute, National Institutes of Health, USA;* [8]*Center for Interventional Oncology, Radiology and Imaging Sciences and Center for Cancer Research, National Institutes of Health, USA*

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Background & Aims: Tremelimumab is a fully human monoclonal antibody that binds to cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) on the surface of activated T lymphocytes. Ablative therapies induce a peripheral immune response which may enhance the effect of anti-CTLA4 treatment in patients with advanced hepatocellular carcinoma (HCC). This study aimed to demonstrate whether tremelimumab could be combined safely and feasibly with ablation.

Methods: Thirty-two patients with HCC were enrolled: male:female: 28:4; median age: 62 (range 36–76). Patients were given tremelimumab at two dose levels (3.5 and 10 mg/kg i.v.) every 4 weeks for 6 doses, followed by 3-monthly infusions until off-treatment criteria were met. On day 36, patients underwent subtotal radiofrequency ablation or chemoablation. Staging was performed by contrast-enhanced CT or MRI scan every 8 weeks.

Results: No dose-limiting toxicities were encountered. The most common toxicity was pruritus. Of the 19 evaluable patients, five (26.3%; 95% CI: 9.1–51.2%) achieved a confirmed partial response. Twelve of 14 patients with quantifiable HCV experienced a marked reduction in viral load. Six-week tumor biopsies showed a clear increase in $CD8^+$ T cells in patients showing a clinical benefit only. Six and 12-month probabilities of tumor progression free survival for this refractory HCC population were 57.1% and 33.1% respectively, with median time to tumor progression of 7.4 months (95% CI 4.7 to 19.4 months). Median overall survival was 12.3 months (95% CI 9.3 to 15.4 months).

Conclusions: Tremelimumab in combination with tumor ablation is a potential new treatment for patients with advanced HCC, and leads to the accumulation of intratumoral $CD8^+$ T cells. Positive clinical activity was seen, with a possible surrogate reduction in HCV viral load.

Lay summary: Studies have shown that the killing of tumors by direct methods (known as ablation) can result in the immune system being activated or switched on. The immune system could potentially also recognize and kill the cancer that is left behind. There are new drugs available known as immune checkpoint inhibitors which could enhance this effect. Here, we test one of these drugs (tremelimumab) together with ablation.

Clinical trial number: ClinicalTrials.gov: NCT01853618.

Keywords: Immune checkpoint; Hepatocellular carcinoma; Immune; T-Lymphocytes; Liver cirrhosis.

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* Corresponding author. Address: National Cancer Institute, 9000 Rockville Pike, 10/12N224, Bethesda, MD 20892, USA. Tel.: +1 (301) 451 4723, fax: +1 (301) 480 8780.

E-mail address: tim.greten@nih.gov (T.F. Greten).

Introduction

Hepatocellular carcinoma (HCC) is one of the most frequently occurring cancers worldwide, ranked 3rd in global incidence by the International Agency for Research on Cancer [1]. HCC typically occurs in the setting of chronic inflammation, such as that induced by viral hepatitis. In contrast to other types of cancer, where surgery, radiation and chemotherapies dominate the therapeutic landscape, in HCC locoregional treatments are widely applied, either with curative (ablative procedures, surgery) or palliative (arterial chemoembolization) intent [2]. Systemic

treatments have a comparatively modest role, sorafenib being the only drug to have demonstrated a survival benefit at the phase III level in the modern era [3,4]. There are several characteristics relating to HCC, which make it amenable to immunotherapy [5]. Spontaneous immune responses including T cell responses, as well as humoral responses to different tumor-associated antigens have been described [6,7]. Interestingly, both transcatheter arterial chemoembolization (TACE) and ablation (either cryo- [CA], microwave [MVA] or radiofrequency [RFA]) by themselves have been shown to induce a peripheral immune response [7–12].

Tremelimumab is a fully human monoclonal antibody that binds to cytotoxic T-lymphocyte-associated protein (CTLA)-4 and results in inhibition of B7-CTLA-4-mediated downregulation of T cell activation. Tremelimumab is well tolerated when administered as a single agent to patients with HCC [13]. The primary aim of this study was to demonstrate whether tremelimumab could be administered safely and feasibly with TACE, RFA or CA. Whilst RFA and cryoablation (CA) procedures are generally employed in early stage disease, here they were employed subtotally in the advanced setting, the hypothesis being that peripheral immune stimulation induced by the ablative procedure could be amplified by immune checkpoint blockade.

Patients and methods

Patients

Eligible patients were at least 18 years old and had histopathological confirmation of hepatocellular carcinoma (HCC) by the Laboratory of Pathology of the National Cancer Institute (NCI) prior to entering this study. Other eligibility criteria included: Eastern Cooperative Oncology Group (ECOG) performance status score 0–2; disease not amenable to potentially curative liver transplantation, resection or ablation. Patients with Barcelona Clinic Liver Cancer (BCLC) Stage C must have had disease amenable to subtotal ablation in addition to having progressed on or been intolerant of prior sorafenib; BCLC Stage B patients were treated with TACE as per the standard of care; Child-Pugh A or B (no more than 7 points) classification if cirrhosis present; no history of chronic autoimmunity or inflammatory bowel disease. All patients provided written informed consent and the study was approved by the NCI Institutional Review Board. The ClinicalTrials.gov identifier was: NCT01853618.

Study design

Patients who satisfied the eligibility criteria were enrolled on a pilot study of tremelimumab at two dose levels (3.5 and 10 mg/kg i.v.) given every 4 weeks for a total of 6 doses, followed by 3-monthly infusions until off-treatment criteria were met (Supplementary Fig. 1). On day 36 (± 96 h) patients underwent subtotal RFA or CA. Subtotal ablation means the complete treatment of a single lesion in the setting of multifocal disease, leaving other lesions (both intrahepatic and extrahepatic) intact and untreated. The lesion subjected to ablation was treated with full ablative intent and chosen at the discretion of the interventional radiologist based on technical factors, such as ease of access, proximity to vessels etc. BCLC B patients received TACE as per standard of care. Staging was performed by contrast-enhanced CT or MRI scan every 8 weeks. Objective response was evaluated in lesions not subject to ablation or TACE using the Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1 criteria. Due to the delayed timing of the radiologic procedure the evaluation period for dose-limiting toxicities (DLT) was extended for the first 8 weeks of the study. The primary objective was to determine if it was feasible to administer tremelimumab in combination with locoregional therapy in patients with advanced HCC. Secondary objectives included a variety of immunologic parameters to determine if there was an immune response to treatment, and to assess safety, toxicity and preliminarily efficacy. To demonstrate feasibility, it was desirable if the fraction of the initial 20 patients receiving 10 mg/kg who could receive all doses of tremelimumab were consistent with 80% or higher. Safety and toxicity were also evaluated and addressed by tabulating and monitoring the grades of toxicity experienced by patients in the study. Feasibility – the primary endpoint – was established in two small pilot cohorts of up to 10 patients each, based on disease stage and/or type of ablation (advanced HCC receiving RFA or TACE; intermediate HCC receiving TACE). The study was then amended to allow additional recruitment to the TACE and ablation cohorts in order to obtain a preliminary assessment of efficacy. Given that these were all small pilot cohorts, no direct comparison was made regarding relative benefits of ablative method or TACE.

Immune correlative studies

All patients on dose level 2 (10 mg/kg) were requested to undergo optional tumor biopsy at the time of the interventional radiologic procedure. Patients underwent blood sampling every cycle with isolation of peripheral blood mononuclear cells (PBMC). T cell subsets were analyzed by multicolor flow cytometry. Activated T cells were analyzed by staining for CD3, CD4, CD8, CD38 and HLA-DR as previously described [14]. Formalin-fixed paraffin-embedded tissue was stained with antibodies for T cell markers CD3 and CD8 (cytotoxic phenotype). Slides were digitally scanned (Aperio ScanScope XT) and analyzed using an automated image analysis algorithm (Aperio Positive Pixel Count v9). The percentage of total positive pixels (corresponding to 3, 3-diaminobenzidine chromogen saturation) in areas of tumor was evaluated between patients stratified by response and pre- and post-treatment biopsies.

Hepatitis monitoring

Patients with HCC in the context of active or chronic hepatitis as well as non-hepatitis etiology were enrolled onto the study. Patients with hepatitis C virus (HCV) were eligible for inclusion whether they had received prior treatment or not. In these patients, HCV viral load was measured every 4 weeks. Patients with chronic hepatitis B were required to be on anti-viral medication. In these patients, hepatitis B virus (HBV) viral load, anti-HBc antibody, anti-HBe antibody, HBeAg and quantitative hepatitis B surface antigen were measured using standard assays every 4 weeks. Serum HBsAg titers were measured by enzyme immunoassay (EIA) using the ARCHITECT platform (Abbott Laboratories, Chicago, IL), as per the manufacturer's instructions.

Safety

All adverse events and serious adverse events occurring within 30 days of the last dose were reported according to the NCI Common Terminology Criteria for Adverse Events v4.0.

Statistical methods

Efficacy was assessed by response rate, as determined by RECIST 1.1 criteria, and reported along with an exact 95% confidence interval. In addition, the time to tumor progression (TTP) and overall survival (OS) were calculated by the Kaplan-Meier method, and reported along with 95% confidence intervals. TTP was the time from consent until the first documented progression of disease. The actuarial probabilities associated with TTP were referred to as tumor progression free survival probabilities. Regarding the immune correlative studies, the actual levels of changes of immune parameters were determined, and the fraction noted to have a change in the parameter values reported. Wilcoxon signed rank test was used to compare T cell subsets at baseline with samples obtained after 4 and 12 weeks. These were considered secondary and exploratory analyses.

Results

Patient characteristics

Thirty-two patients were enrolled onto this pilot study evaluating immune checkpoint inhibition with tremelimumab in combination with either RFA, CA or TACE (Table 1). Four patients experienced rapid disease progression in the first few weeks of study and were evaluated for safety only. The baseline characteristics for the study population patients are shown in Table 1. The median age of the population was 61 (range 36–76). Cirrhosis