Published in final edited form as:

Hepatology. 2019 February ; 69(2): 831–844. doi:10.1002/hep.30222.

Liver complications following treatment of hematologic malignancy with ANTI-CD22-calicheamicin (inotuzumab ozogamicin)

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Abstract

Background and Rationale: Treatment of hematologic malignancy with antibody-drug conjugates (ADC) may cause liver injury. ADCs deliver a toxic moiety into antigen-expressing tumor cells but may also injure hepatic sinusoids (Sinusoidal Obstruction Syndrome, SOS). We studied patients who received an anti-CD22-calicheamicin conjugate (inotuzumab ozogamicin) to gain insight into mechanisms of sinusoidal injury, as there are no CD22+ cells in the normal liver but non-specific uptake of ADCs by liver sinusoidal endothelial cells.

Methods: 638 patients (307 with acute lymphocytic leukemia (ALL), 311 with non-Hodgkins lymphoma) were randomized to either inotuzumab ozogamicin or standard chemotherapy (controls). While blinded to treatment assignment, we reviewed all cases with hepatobiliary complications to adjudicate the causes.

Results: The frequency of SOS among patients who received inotuzumab ozogamicin was 5/328 (1.5%), compared to no cases among 310 control patients. Drug-induced liver injury (DILI) developed in 26 (7.9%) inotuzumab ozogamicin recipients and 3 (1%) controls. Intrahepatic cholestasis was seen in 4.9% of inotuzumab ozogamicin recipients and in 5.5% of controls. Subsequent to the randomization study, 113 patients with ALL underwent allogeneic hematopoietic cell transplantation; the frequency of SOS in those previously exposed to InO was 21/79 (27%) vs. 3/34 (9%) in controls. An exploratory multivariate model identified a past history of liver disease and thrombocytopenia before conditioning therapy as dominant risk factors for SOS after transplant.

Conclusions: The frequencies of SOS and DILI after inotuzumab ozogamicin treatment were 1.5% and 7.9%, respectively, compared to none and 1% among controls who received standard chemotherapy. These data suggest that ADCs that do not target antigens present in the normal liver have a relatively low frequency of SOS but a relatively high frequency of DILI.

Keywords

Liver; drug-induced liver injury; antibody-drug conjugates; sinusoidal obstruction syndrome; hepatic veno-occlusive disease; cholestasis; acute lymphocytic leukemia; non-Hodgkins lymphoma; CD22; CD33; calicheamicin; hematopoietic cell transplantation

Introduction

The advent of antibody-drug conjugates (ADC), designed to deliver a toxic moiety into tumor cells, has altered the approach to many cancers, particularly hematologic malignancies. One of the putative advantages of targeted therapy is the avoidance of toxicity to normal cells and thus, a lowering of the frequency of systemic side effects, compared to non-targeted standard chemotherapy regimens. However, unexpected toxicity has been seen after ADC therapies, through several potential mechanisms: expression of the antigenic target by normal tissues, innocent bystander effects related to organ involvement by tumor, uptake of ADCs by liver sinusoidal endothelial cells (LSEC), and disposition of the toxic moiety resulting in damage to excretion pathways.(1) (2) (3) (4)

An example of unexpected toxicity was seen after treatment of acute myeloid leukemia with the CD33 antigen-targeted immunoconjugate N-acetyl dimethyl hydrazide calicheamicin (gemtuzumab ozogamicin, GO, Mylotarg™), which caused liver toxicity.(5) (6) The presentation of this toxicity, however, did not fall into a standard classification of Drug-Induced Liver Injury (DILI) but rather presented with signs and symptoms of acute portal hypertension, caused by damage to liver sinusoidal endothelial cells (LSECs), embolization of LSECs and hepatocytes, activation of stellate cells, and deposition of collagens into liver sinusoids, resulting in obstruction to sinusoidal blood flow.(7) (8) The resulting Sinusoidal Obstruction Syndrome (SOS, formerly known as Veno-Occlusive Disease of the liver) was similar in its presentation and course to acute sinusoidal injury caused by regimens of high-dose chemotherapy and irradiation used to prepare patients for hematopoietic cell transplantation (HCT).(9) (10) A prominent histologic feature of GO-caused SOS is deposition of collagens within sinusoids, a result of activation of hepatic stellate cells.(7) (11) Stellate cells are activated when sinusoidal endothelial cells are damaged, resulting in loss of a signal that keeps stellate cells in a quiescent state.(12)

Inotuzumab ozogamicin (InO), an immunoconjugate with the same calicheamicin moiety as gemtuzumab ozogamicin but targeted at leukemia and lymphoma cells expressing CD22, has the potential to cause the same sort of sinusoidal liver damage as gemtuzumab ozogamicin, but with one significant difference: there are no cells in the normal liver that express CD22, with the exception of lymphocyte populations resident in the liver (mostly confined to portal areas). If SOS that occurs after gemtuzumab ozogamicin is related to targeting of CD33-expressing cells normally resident in hepatic sinusoids, the frequency of SOS after therapy with InO should be significantly less. However, because hepatic sinusoids are often a reservoir of leukemic cells in patients with refractory CD22+ acute lymphocytic leukemia (ALL), and portal areas are frequently infiltrated with CD22+ lymphoma cells in patients with non-Hodgkins lymphoma (NHL), the opportunity exists for a bystander effect on the liver following treatment with InO.(13) (14) (15) Alternatively, if endocytosis of

ADCs via the Fc γ RIIb2 receptor on LSECs is the proximate cause of LSEC damage, the frequency of SOS following InO therapy could be similar to that following GO therapy, even though there are no CD22+ cells in normal hepatic sinusoids, except for CD22+ malignant cells. These are not mutually exclusive mechanistic hypotheses.

In the studies reported here, a panel of hepatologists familiar with DILI and sinusoidal liver injury reviewed all subjects with liver abnormalities following InO therapy in two multicenter protocols. One study randomized patients with relapsed or refractory ALL to single agent InO versus one of three investigator-choice standard chemotherapy regimens, as previously described.(16) The other study randomized patients with Non-Hodgkin's Lymphoma (NHL) to InO plus rituximab vs. an investigator-choice standard chemotherapy regimen.(17) Liver abnormalities were categorized by our panel (blinded to treatment assignment) into one or more diagnostic categories (Table 1) on the basis of clinical presentation, laboratory and imaging tests, proximity to study drug, clinical course, and liver histology, when available. We categorized the causes of liver abnormalities in the following sub-cohorts of patients: InO-treated patients with ALL; InO-treated patients with NHL; patients with ALL treated with standard chemotherapy; and patients with NHL treated with standard chemotherapy.

Methods

Patient selection.

All subjects with either ALL or NHL who were randomized and treated according to randomization assignment (InO vs. standard therapy control) were eligible for review of adverse events involving the liver. There were two separate randomized, phase 3, multicenter studies sponsored by Pfizer Incorporated (New York, NY). Protocol B1931008 (formerly Wyeth 3129K5–3303-WW; NCT01232556) evaluated the efficacy and safety of InO, a CD22-targeted antibody linked to the toxic agent calicheamicin, in combination with rituximab in subjects with relapsed/refractory aggressive NHL, compared to an active control arm consisting of the investigator's choice between two rituximab (R)-containing chemotherapy regimens, R-bendamustine or R-gemcitabine.(17) Study B1931022 (NCT01564784, the INO-VATE study) evaluated the efficacy of InO in an open-label trial in subjects with relapsed or refractory CD22+ ALL, compared to an active control group of subjects who received one of three regimens chosen by study site investigators (FLAG, fludarabine, cytarabine and G-CSF for up to 4 cycles; or cytarabine and mitoxantrone for up to 4 cycles; or HIDAC, high dose cytarabine for up to 12 doses per cycle for up to 2 cycles). (16) In these trials, InO was given intravenously for up to 6 cycles at a dose of 1.8 mg/m² per cycle (potential maximum InO exposure, 10.8 mg/m²), as previously described.(16) Once complete remission was achieved, InO dose was decreased to 1.5 mg/m²/cycle for all other cycles. In Study B1931022, InO was given in split/weekly doses (3 doses per cycle) while in Study B1931008, InO was given as a single dose per cycle. The majority of patients in the InO arm received 1 to 4 cycles of treatment. In the control arms, majority of patients received 1 to 2 cycles of treatment. Study subjects signed informed consent documents approved by Institutional Review Organizations at each study site.

Monitoring of adverse events involving the liver.

Hepatobiliary adverse events were identified when either liver-related adverse events were reported by a clinical site investigator or identified by the Pfizer Study Team during periodic data review. There were 2 “study periods” during which Pfizer gathered data about hepatobiliary complications. The first period included events during study treatment (with either InO or the control standard chemotherapy) and included events through 28 days after the last dose of study drug or the end-of-treatment visit, whichever occurred later. The second period included any study treatment-related events after this for up to 2 years after randomization. Potential hepatobiliary events included, but were not limited to the following: abnormal serum liver tests (alanine and aspartate aminotransferases, alkaline phosphatase, gamma glutamyl transpeptidase, total serum bilirubin); clinical diagnoses of hepatic veno-occlusive disease or SOS, nodular regenerative hyperplasia, liver failure, multi-organ failure, cirrhosis or hepatic fibrosis, right upper quadrant pain, abdominal pain thought to be hepatobiliary in origin, ascites, hepatomegaly, and any event that fell under the System Organ Class MedDRA query for hepatobiliary disorders. For each subject identified as having a liver-related adverse event, the Pfizer Study Team prepared a dossier of data that was de-identified as to randomization assignment, consisting of 1) a summation of Serious Adverse Event forms; 2) subject narrative (demography, medical history, prior therapy for the underlying malignancy including conditioning therapy for HCTs, hepatic history, surgical history, non-study medications - including herbal supplements and herbal teas -- vital signs, changes in weight, results of physical examination, laboratory values, sites of malignant disease, other adverse events, and other non-study treatments); 3) start dates of each study drug cycle (study drug was not identified); 4) hospital discharge summary and other relevant medical/hospital records such as consultation reports; and 5) all relevant diagnostic test and imaging results, if performed and provided by the study site, including but not limited to CT, MRI, Doppler ultrasound, tissue biopsy, and autopsy reports. For non-English language reports, original untranslated versions, as well as English language translated versions, were provided.

Role of Hepatic Events Adjudication Boards (HEABs).

A Hepatic Events Adjudication Board (HEAB) was established for each InO study as an external, independent, expert advisory group to oversee the safety of InO with respect to hepatobiliary events. For Study B1931008, HEAB members included Drs. DeLeve, McDonald, and Freston; for Study B1931022, Drs. Boyer, McDonald, and Freston. For subjects identified as having liver disorders as adverse events, dossiers were distributed to HEAB members in advance of regularly scheduled telephone conferences that encompassed both open and closed sessions, the former including brief case presentations by the Pfizer Study Team and the latter a confidential discussion among all HEAB reviewers (Drs. DeLeve and McDonald for Study B1931008, Drs. Boyer and McDonald for Study B1931022, with Dr. Freston as the tie-breaker for both studies in the event of disagreement as to classification). HEAB deliberations for each subject resulted in one or more liver disease classifications as shown in Table 1, that is, the classifications were not mutually exclusive. The classification ‘other hepatic disease’ was accompanied by a description as to the nature of the liver disorder. The classification ‘not a hepatic event’ was applied to situations where the signs, symptoms, and laboratory result were not of liver origin, for

example, abdominal pain of gastrointestinal and not liver origin. The classification ‘not enough information to classify’ was applied when there was a paucity of clinical data to explain a liver-related abnormality. When the HEAB classified a subject as having SOS, it was accompanied by an opinion as to certainty of diagnosis (definite, probable, possible) and severity (mild, moderate, severe). When the HEAB identified more than one hepatobiliary adverse event during the study period, the most serious diagnosis was identified by Dr. McDonald according to a rule-based system: SOS was listed as the most serious diagnosis whenever it was identified. Otherwise, the diagnosis with the highest total serum bilirubin in closest proximity to assigned therapy was identified and carried forward as the classification in statistical analyses.

Statistical methods.

The frequency of liver-related adverse events in both studies was analyzed separately for events that occurred after randomization and the assigned treatment, and events that occurred after a subsequent conditioning regimen (also known as the preparative regimen) and infusion of donor cells (hematopoietic cell transplantation). The frequency of occurrence of liver disease classifications (as determined by the HEAB) in the InO arm was compared to that in the control arm by Fisher’s Exact test. For the subset of patients with ALL enrolled in Study B1931022 who subsequently underwent HCT, univariate and multivariate analyses were conducted to identify potential risk factors for post-transplant development of SOS. In the univariate analysis, for each potential risk factor, the frequency of the categorical variable or distribution of the continuous variable was compared between patients who developed SOS after HCT and those who did not develop SOS after HCT. P-values from Fisher’s Exact test and Wilcoxon rank-sum test were reported for categorical variables and continuous variables, respectively. Factors with a p-value <0.10 in the univariate analysis were included in the multivariate logistic regression modeling using stepwise selection. A significance level of 0.3 was required to allow a variable into the model and a significance level of 0.1 was required for a variable to stay in the model.

Results

Liver disorders that occurred during and after treatment on protocol, before hematopoietic cell transplantation

Liver disorders in patients with ALL (Table 2)—The overall frequency of liver disorders in patients with ALL (Study B1931022) as reviewed by the HEAB was numerically but not significantly greater in patients randomized to receive InO than in patients receiving a standard chemotherapy regimen (22.6% vs. 16.8%, $p=0.2515$) (Table 2). In the InO arm, there were 2 cases of severe SOS (one scored as definite, one as probable) and 1 case of mild SOS, scored as probable; no cases of SOS were found in the standard chemotherapy arm (1.8% vs. 0, $p=0.2511$). There were significantly more cases of DILI (that did not meet criteria for SOS) in the InO arm, compared to the standard chemotherapy arm (7.9% vs. 1.4%, $p=0.0079$). The frequency of other liver disorders was not significantly different in the two arms, including intrahepatic cholestasis (6.1% in the InO arm vs. 11.2% in the standard chemotherapy arm, $p=0.1495$). Most of the cases of cholestasis were in the setting of febrile neutropenia and infection.

Liver disorders in patients with NHL (Table 2)—The overall frequency of liver disorders was significantly greater in patients randomized to receive InO-R than in patients receiving standard chemotherapy (26.8% vs. 7.8%, $p=0.0001$) (Table 2). In the InO-R arm, there were 2 cases of SOS (one scored as possible and mild, one as possible and moderate); no cases of SOS were found in the standard chemotherapy arm (1.2% vs. 0, $p=0.2447$). In the InO-R arm, there were significantly more cases of DILI, compared to the standard chemotherapy arm (7.9% vs. 0.6%, $p=0.0007$), and more cases classified as ‘other hepatic disease’ (9.1% vs. 2.4%, $p=0.0091$). The disorders covered by the ‘other hepatic disease’ classification encompassed Gilbert’s syndrome and minor elevations of total serum bilirubin or liver enzymes remote from treatment periods that did not meet criteria for DILI (see footnotes e and f, Table 2). The reason for the greater frequency of ‘other hepatic disease’ in recipients of InO-R is not readily apparent, but it is possible that this category includes some instances of DILI with a delayed appearance. The frequency of cirrhosis, non-infectious hepatitis, intrahepatic cholestasis, and biliary obstruction was not significantly different in the two arms.

Comparison of the frequency of specific liver disorders in InO-treated subjects, by underlying diagnosis (ALL vs. NHL), before subsequent HCT (Table 2)—During study therapy, the overall frequency of liver disorders was similar among InO recipients with ALL vs. InO-R recipients with NHL (22.6% vs. 26.8%, $p=0.4425$), and there were similar frequencies of SOS (1.8% vs. 1.2%), DILI (both 7.9%), cholestasis (6.1% vs. 3.7%) and other disorders (Table 2). Severe SOS was seen in two subjects with ALL who received InO; there were no severe SOS cases among subjects with NHL who received InO.

Liver disorders in patients with ALL, after subsequent conditioning therapy and hematopoietic cell transplantation

Comparison of the frequency of specific liver disorders by randomization arm (InO vs. standard chemotherapy) (Table 3)—After randomization and treatment on Study B1931022, 79 subjects who had received InO and 34 subjects who had received only standard chemotherapy, including additional salvage therapy in some patients, proceeded to conditioning therapy (also called the preparative regimen, consisting of chemotherapy drugs with or without total body irradiation) for allogeneic HCT. The overall frequency of liver disorders was greater in patients previously exposed to InO prior to subsequent HCT than in patients who had previously received standard chemotherapy (34% vs. 12%, $p=0.0203$) (Table 3). The overall frequency of SOS in those previously exposed to InO was 21/79 (27%) vs. 3/34 (9%) in those previously exposed to standard chemotherapy ($p=0.0442$). Moderate to severe SOS developed after HCT in 19/79 (24%) InO-exposed subjects, compared to 3/34 (9%) subjects who had previously received only standard chemotherapy ($p=0.0726$).

Risk factors for development of SOS in patients with ALL treated on Study B1931022 (Tables 4 and 5)—We examined potential risk factors for development of SOS after HCT according to demographic factors, past medical history, time of randomization-to-HCT factors, and factors related to the HCT procedure, including

conditioning therapy, donor type, and GVHD prophylaxis. By univariate analysis, factors associated with development of SOS after HCT at the $p < 0.05$ level of significance included older age ($p = 0.0435$); HCT prior to study therapy ($p = 0.0230$); history of liver disease before study entry ($p = 0.0073$); exposure to InO ($p = 0.0442$); alkaline phosphatase level during the study period and just before conditioning therapy ($p = 0.0004$ and $p = 0.0001$, respectively); and low leukocyte and platelet counts just before conditioning therapy ($p = 0.0439$ and $p = 0.0004$, respectively) (Table 4). A multivariate model was developed in which all factors related to development of SOS after HCT with a p -value < 0.10 based on univariate analysis were considered for entry. After stepwise selection, factors that remained in the final model included HCT prior to study therapy, history of liver disease, alkaline phosphatase level before conditioning therapy, thrombocytopenia before conditioning therapy, and GVHD prophylaxis with a calcineurin inhibitor and methotrexate. Among these factors, history of liver disease (odds ratio: 3.498, 95% Confidence Interval [CI] 1.065 – 11.492) and thrombocytopenia before start of conditioning therapy (odds ratio: 4.223, 95% CI 1.279–13.939) were associated with a p -value < 0.05 . The multivariate analysis confirmed observations in the univariate analysis and suggested that these two factors may be associated with a higher risk of SOS after HCT. Risk factors that were significant in the univariate analysis that did not enter the multivariate model were prior exposure to InO, older age, alkaline phosphatase levels during the study period, and low leukocyte counts. However, given that the analysis was done in a relatively small sample size without multiplicity adjustment, we consider these results to be suggestive and hypothesis-generating.

Discordant liver diagnoses in patients with ALL, as made by study site investigators versus HEAB hepatologists.

Most of the Study B1931022 cases reviewed by the HEAB because of hepatobiliary problems had been reported by study site investigators with descriptive terms such as hyperbilirubinemia, elevated liver enzymes, liver test abnormalities, liver dysfunction, and ascites, without providing an underlying diagnosis. Two subjects who received InO and whose liver problem was termed ‘ascites’ by investigators were classified by HEAB as having intra-abdominal tumor and pancreatic ascites, respectively. Of six subjects whose liver problems after randomization were given specific diagnoses by study site investigators, two were discordant with HEAB diagnoses, both termed VOD by investigators but classified as intrahepatic cholestasis by the HEAB (Table 6). Of twenty-three subjects in whom study investigators had reported specific liver diagnoses following HCT, three were discordant with HEAB diagnoses. One discordant case, previously exposed to InO, carried an investigator diagnosis of VOD that the HEAB classified as intrahepatic cholestasis plus non-alcoholic steatohepatitis. Two discordant cases, both previously exposed to InO, were discordant only in a technical sense (investigator diagnoses were ‘hepatotoxicity’ and ‘multi-system dysfunction’ -- both were HEAB-classified as SOS).

Discussion

Liver toxicity, specifically injury to hepatic sinusoids, is a major concern for development of antibody-drug conjugates.⁽⁴⁾ There are four potential mechanisms for hepatic injury from

ADCs – 1) targeting of normal sinusoidal cells that express the same antigen as expressed on malignant cells; 2) uptake of ADCs via endocytosis by LSECs and Kupffer cells; 3) presence of malignant cells in hepatic sinusoids that express the antigen of interest, resulting in release of toxin into sinusoids after apoptosis of malignant cells; and 4) damage to disposition pathways caused by exposure to a metabolite of the toxic moiety. The ADC inotuzumab ozogamicin is a CD22-targeted humanized immunoglobulin type G, subtype 4 (IgG4) antibody covalently linked to N-Ac- γ -calicheamicin dimethylhydrazide. There is intracellular release of the cytotoxic component (N-Ac- γ -calicheamicin dimethylhydrazide) which then is metabolized by non-enzymatic reduction, followed by biliary elimination. These are not mutually exclusive mechanisms for liver injury following ADCs.

The major findings by our panel of hepatologists from the two randomized trials of single agent InO or InO-R versus standard chemotherapy drug regimens for the malignant disorders ALL and NHL are the following:

1. The combined frequency of SOS (all degrees of severity and certainty of diagnosis) among patients who received InO was 5/328 (1.5%), compared to no cases among 310 patients who received chemotherapy regimens (Table 2). Among 638 randomized patients, severe SOS was seen only in 2 ALL patients following InO treatment (0.3%).
2. The overall frequency of SOS was similar between those treated with InO for ALL vs. those treated with InO-R for NHL (Table 2).
3. A more frequent liver complication among patients who received InO was DILI (Table 2). The frequency of DILI in ALL study participants was significantly more frequent among recipients of InO (7.9%) than among those randomized to standard chemotherapy alone (1.4%) ($p=0.0079$). In the NHL study, similar increased frequencies of DILI were seen (7.9% in the InO group vs. 0.6% in the standard chemotherapy group, $p=0.0007$). For the most part, the manifestations of DILI were transient and asymptomatic elevations of serum ALT, alkaline phosphatase, or total serum bilirubin, without evidence of portal hypertension, weight gain, or fluid accumulation. We could not determine with any certainty whether DILI was related to InO exposure or toxicity from concomitant medications or from hepatic effects of concomitant infection, much less to define the exact drug that was responsible for DILI. For example, most patients in both arms of both protocols received antibiotics, antifungal drugs, and antiviral drugs, as prophylaxis, pre-emptive coverage (especially for viral disease), and treatment for infection. We can only speculate that the gross imbalance in the frequency of DILI in the InO arms of two large trials represents a causal relationship.
4. Another frequent liver complication in both arms of the ALL study was intrahepatic cholestasis, observed in 6.1% and 11.2% of InO and standard chemotherapy recipients, respectively ($p=0.1495$). Intrahepatic cholestasis was less frequent in the NHL study. Almost all cases of intrahepatic cholestasis were in the setting of systemic infection, usually sepsis, abscess, or protracted neutropenic fever.

Oncologists who enrolled patients with ALL in the B1931022 study (the INO-VATE study) have published on hepatic adverse events as recorded by study site investigators.(18) While the general conclusions of their analysis (“Treatment with InO is associated with increased hepatotoxicity, especially after follow-up HSCT, compared with standard care”) is concordant with our analysis, our panel determined that the majority of adverse liver events were caused by DILI or intrahepatic cholestasis and not by hepatic sinusoidal injury (SOS). In subjects with a DILI classification, the offending drug (InO vs. standard chemotherapy vs. concomitant medication such as an antimicrobial drug) could not be identified with any certainty. We speculate that disposition of a calicheamicin metabolite by hepatocytes and subsequent biliary excretion is a possible mechanism for DILI in InO recipients, but our data do not address this hypothesis. Almost all cases of intrahepatic cholestasis were related to infection, in turn related to the effect of inflammatory cytokines (interleukin-6 and TNF α) on ABC2 hepatocyte transporters of bilirubin diglucuronide.(19, 20) (21) The data show that while the frequency of SOS in these studies was low, this diagnosis was made only in patients who had received InO (frequency 1.5%), and the only severe SOS cases were in ALL study patients who had received InO. Several diagnoses of SOS by study site investigators were unable to be confirmed by our panel of hepatologists. We had the advantage of being blinded to study assignment but the disadvantage of not being at the bedside to examine patients or liver histology in the few patients who had tissue available. Our exploratory analysis of risk factors for SOS in study patients with ALL who subsequently underwent HCT found that prior InO exposure was one of several risk factors, but not the dominant factor in an exploratory multivariable analysis. InO exposure had not been examined as a potential risk factor for post-transplant SOS in the study site analysis. (18)

These data from patients with hematologic malignancy receiving InO stand in contrast to a greater reported frequency of SOS and severe SOS in patients exposed to gemtuzumab ozogamicin (GO), an anti-CD33 ADC with the same toxic moiety (calicheamicin) as InO. The frequency of SOS following GO monotherapy varies with the cumulative dose, estimated at 3% when the GO dose was 6mg/m² and 15% at a GO dose of 9 mg/m².(22) In the clinical setting where GO is combined with other chemotherapy drugs or in proximity to myeloablative conditioning therapy, the frequency of SOS can be higher.(6) (22) In study B1931022, the median overall dose of InO in patients with ALL was 4.2 mg/m², and the frequency of severe SOS was 2/164 (1.2%). A comparison of the frequency of sinusoidal liver toxicity in patients who receive InO vs. GO is complicated by both different dosing schedules and the greater amount of calicheamicin in InO compared to GO. We speculate that greater sinusoidal liver toxicity from GO monotherapy, compared to that following InO exposure, is related to the expression of CD33⁺ cells resident in the normal liver, compared to the absence of CD22⁺ cells in the normal liver. Calicheamicin delivery to sinusoidal endothelial cells and Kupffer cells that express CD33 is the likely mechanism behind the apparently greater sinusoidal toxicity of GO.(23) (24)

Does exposure to InO increase the risk of developing SOS after a subsequent myeloablative conditioning regimen and allogeneic HCT? Data from Table 3 suggests that InO exposure in patients with ALL may increase the risk of SOS after a myeloablative conditioning regimen (21 of 79 patients who had been randomized to the InO arm developed SOS after HCT,

compared to 3 of 34 patients from the chemotherapy arm, $p=0.0442$). Moderate and severe SOS cases were also more frequent in patients from the InO arm. However, exposure to InO was not the only risk factor for development of SOS after HCT by univariate analysis and failed to enter the final multivariate model after stepwise selection, which identified a history of liver disease before randomization and thrombocytopenia before conditioning therapy as the dominant risk factors for post-HCT SOS. Although InO exposure itself was not significant in the multivariable model, it is possible that InO played a role in causing thrombocytopenia in the time period before HCT, as delayed platelet recovery is a recognized effect of InO therapy.

The mechanisms by which InO might increase the risk of post-transplant SOS have been clarified by development of a primate model of ADC-related sinusoidal liver injury.(25) In this study, a non-binding antibody-calicheamicin conjugate was administered to cynomolgus monkeys at doses equivalent to human exposures to GO and InO. At day 3 after exposure, there was loss of liver sinusoidal endothelial cells and mid-zonal hepatocyte degeneration; at day 63, variable LSEC recovery and sinusoidal capillarization and dilation were seen. While this study points to uptake of the antibody-calicheamicin conjugate as one mechanism of LSEC injury, it begs the question as to whether the results would be different if InO or GO were infused.

If InO is to be used as a bridge to allogeneic transplant in patients with ALL, future protocols should be directed at lessening the impact of myeloablative conditioning therapy on cells in the hepatic sinusoids. There are two general approaches: cytoprotection of LSECs and modifications to conditioning regimens that are known to damage LSECs. Although small animal studies of toxin injury have identified potential LSEC cytoprotectants(26) (27) (28), clinical studies have not been done, and there is concern that LSEC cytoprotection methods might cytoprotect malignant cells and lead to worse outcomes. Conditioning regimen modifications to be considered include the choice of components of these regimens -- avoiding known LSEC toxins (cyclophosphamide, etoposide, and higher-dose total body irradiation, for example); therapeutic drug monitoring to avoid exposures to high-levels of toxic metabolites; optimizing the order of multidrug regimens; and limiting doses of total body irradiation.(29) (30) (31) (32)

Only one therapy (defibrotide) has been FDA- and EMA-approved for the indication SOS. However, the majority of defibrotide-treated patients on which approvals were based did not receive antibody-drug conjugates such as GO and InO. Description of liver histology at various stages of SOS following GO and InO is limited. One GO report noted intense deposition of collagen within sinusoids, without much zone 3 hemorrhage, suggesting that stellate cell activation and matrix production was a dominant feature of GO-caused sinusoidal injury.(33) Because the effectiveness of defibrotide in patients with ADC-related sinusoidal injury is unknown, and because of the cost (exceeding \$100,000 USD currently), having a certain diagnosis of SOS before starting therapy would seem wise. There are mimics of SOS that are difficult to differentiate from SOS, particularly sepsis syndrome with hypotension and subsequent liver and renal dysfunction; cardiac failure with passive congestion in the liver, hypoxic hepatitis, and renal insufficiency; cholestatic liver disease related to the cytokines interleukin-6 and TNF α ; pre-existing hepatic fibrosis and cirrhosis

with decompensation related to post-transplant liver insults; and combinations of these disorders.(29) The most accurate diagnostic tools are Doppler ultrasound (detection of ascites, measurement of resistive indices to hepatic artery flow, direction of portal venous flow) and a transvenous approach via the hepatic vein for measurement of the wedged hepatic venous pressure gradient using the Groszmann method and liver biopsy histology.(7) (34) Transvenous liver biopsy in experienced hands can be done safely in patients whose platelet count is $>30,000/\text{mm}^3$ using either a needle biopsy instrument via a jugular vein or a Mansfield forceps via a femoral vein.(35)

Are there treatment recommendations for patients who develop SOS after InO exposure that can be derived from the data in this paper? In the cohorts of patients with ALL and NHL who received InO, 5 developed SOS during the study period, of whom 3 recovered without specific treatment and 2 died with severe SOS (Table 2). In the cohort of ALL patients who came to subsequent HCT, 21 developed SOS, of whom 12 recovered and 9 died with severe SOS. Once a definitive diagnosis of SOS has been made, estimates of the risk that SOS will be fatal can be based on clinical, laboratory, imaging, and histologic criteria.(29) Defibrotide infusions are approved for the indication severe SOS, but data on effectiveness in treating SOS following ADC-caused SOS is limited. There is anecdotal experience with using pre-emptive hemofiltration to prevent massive extravascular fluid accumulation, particularly in the lungs.(36) The development of a primate model of LSEC injury following InO exposure will provide an opportunity to study prevention and treatment of LSEC damage.(25)

Acknowledgments

Financial support:

Pfizer Inc. (New York, NY) funded two randomized, multicenter studies of inotuzumab ozogamicin that were conducted by oncologists (see citations 16 and 17 in the list of references). Data from these studies were reviewed by the authors who were paid consultants to Pfizer Inc., for the purpose of adjudicating the causes of liver disorders that occurred in study participants. Pfizer Inc. provided the authors with case summaries that were de-identified as to treatment assignment. After the studies were closed and our Hepatic Events Adjudication Boards had completed their tasks of evaluating liver disorders in study participants, the data were unblinded and the authors wrote the manuscript, without additional compensation from Pfizer Inc. The manuscript was written by the co-authors without any editorial assistance from Pfizer or an independent agency. Pfizer Oncology scientific and legal staff reviewed the manuscript for errors of fact. None of the authors has a conflict of interest with regard to the data reported in this manuscript or with regard to Pfizer Inc. We are grateful for the data recorded by study site investigators and for the assistance provided by Pfizer Oncology's study teams (Barbara Sleight, Fausto Loberiza, Christine G. DiRienzo, Sharon Sullivan, Erik Vandendries), their data base access, clinical narratives, and statistical analyses (Hui Zhang).

List of abbreviations:

ADC	antibody-drug conjugate
LSEC	liver sinusoidal endothelial cells
GO	gemtuzumab ozogamicin
DILI	drug-induced liver injury
SOS	sinusoidal obstruction syndrome
HCT	hematopoietic cell transplantation

InO	inotuzumab ozogamicin
ALL	acute lymphocytic leukemia
NHL	non-Hodgkins lymphoma
HEAB	Hepatic Events Adjudication Board

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Table 1.

Hepatobiliary disorder classifications that the Hepatic Event Adjudication Boards used to categorize each subject's case.

Classification	Comments
Sinusoidal Obstruction Syndrome (SOS); formerly known as veno-occlusive disease of the liver (VOD)	Mild: clinically apparent, required no treatment, and resolved completely. Moderate: signs and symptoms required treatment such as diuretics or pain medications, but resolved completely. Severe: required treatment but did not resolve before 100 days or before death.
Drug Induced Liver Injury (DILI)	DILI diagnosis was based on compatible time-to-onset of the injury following start of the drug; improvement after drug discontinuation (de-challenge); exclusion of competing alternative causes; and laboratory findings compatible with DILI (elevated serum alanine aminotransferase (ALT) or aspartate aminotransferase (AST), conjugated bilirubin, or alkaline phosphatase exceeding 2-times the upper limit of normal. Classification of DILI was accompanied by an opinion as to certainty of diagnosis (definite, probable, possible). A diagnosis of DILI did not imply that assigned therapy (InO or a chemotherapy regimen) was the cause, as use of hepatotoxic concomitant medications was frequent.
Cirrhosis / fibrosis	Based on clinical, imaging, and/or histological criteria
Nodular regenerative hyperplasia	Based on clinical and/or histological criteria
Infectious hepatitis	Viral, fungal, or bacterial infection
Non-infectious hepatitis	Alcoholic hepatitis, non-alcoholic steatohepatitis, drug-induced hepatitis
Intrahepatic cholestasis	Hyperbilirubinemia related to injury, inflammation, or cytokine-mediated effects on transport of conjugated bilirubin into bile (sepsis-associated cholestasis)
Extrahepatic biliary obstruction	Obstruction at the level of the porta hepatis, extrahepatic biliary system, or ampulla of Vater
Tumor involvement of hepatobiliary tree	Evidence of malignant cells in the liver or bile ducts by imaging, histology, and/or physical examination
Other hepatic disease	A liver disorder that did not fall into another category
Not a hepatic event	No evidence of hepatobiliary pathology, usually with alternate explanation of clinical or laboratory findings

Table 2.

Hepatic Events Adjudication Board classifications of liver disorders for Studies B1931008 and B1931022, before any conditioning therapy in preparation for hematopoietic cell transplantation.

HEAB classification	Acute Lymphocytic Leukemia		Non-Hodgkins Lymphoma			P-value ^b for comparison of ALL-InO vs. NHL-InO
	InO (n=164)	Standard Chemotherapy ^a (n=143)	P-value ^b	InO (n=164)	Standard of Care (n=167)	P-value ^b
Liver disorder reported after randomization and study therapy						
YES	37 (22.6%)	24 (16.8%)	0.2515	44 (26.8%)	13 (7.8%)	<0.0001
NO	127 (77.4%)	119 (83.2%)		120 (73.2%)	154 (92.2%)	0.4425
SOS-all degrees of certainty, all degrees of severity	3 (1.8%)	0	0.2511	2 (1.2%)	0	1.0
Definite SOS, severe	1	0		0	0	
Probable SOS, severe	1	0		0	0	
Probable SOS, mild	1	0		0	0	
Possible SOS, mild	0	0		1	0	
Possible SOS, moderate	0	0		1	0	
Drug-Induced Liver Injury, all degrees of certainty	13 (7.9%)	2 (1.4%)	0.0079	13 (7.9%)	1 (0.6%)	1.0
Probable DILI	6	0		5	0	
Possible DILI	7	2		8	1	
Cirrhosis	1 (0.6%)	1 (0.7%)	1.0	1 (0.6%)	0	0.4955
Nodular regenerative hyperplasia	0	0	-	0	0	--
Infectious hepatitis	0	0	-	0	0	--
Non-infectious hepatitis, including alcoholic and non-alcoholic steatohepatitis	1 (0.6%)	0	1.0	1 (0.6%)	2 (1.2%)	1.0
Other hepatic disease	4 (2.4%) ^c	4 (2.8%) ^d	1.0	15 (9.1%) ^e	4 (2.4%) ^f	0.0159
Intrahepatic cholestasis	10 (6.1%)	16 (11.2%)	0.1495	6 (3.7%)	1 (0.6%)	0.4430
Extrahepatic biliary obstruction or tumor involvement of biliary tree	4 (2.4%)	1 (0.7%)	0.3768	4 (2.4%)	3 (1.8%)	1.0

HEAB classification	Acute Lymphocytic Leukemia		Non-Hodgkins Lymphoma		P-value ^b for comparison of ALL-InO vs. NHL-InO
	InO (n=164)	Standard Chemotherapy ^a (n=143)	InO (n=164)	Standard of Care (n=167)	
Not enough information for the HEAB to classify the cause of liver disease	1 (0.6%) ^g	0	2 (1.2%) ^h	2 (1.2%) ⁱ	--

^aFludarabine / cytarabine / granulocyte colony-stimulating factor (FLAG); cytarabine / mitoxantrone; or high-dose cytarabine.

^bP-values calculated by Fisher's Exact Test.

^cInO arm: Of the 4 ALL patients classified as 'other hepatic disease', these were the clinical situations:

- Increased unconjugated serum bilirubin caused by Gilbert's Syndrome (n=1)
- Budd-Chiari syndrome, later development of refractory GVHD with liver involvement (n=1)
- Following sepsis syndrome, development of hypoxic hepatitis and cholestasis of infection (n=1)
- Increased unconjugated serum bilirubin from hemolysis, minimal liver enzyme elevation, cause unknown (n=1)

^dControl arm: Of the 4 ALL patients classified as 'other hepatic disease', these were the clinical situations:

- Liver toxicity caused by ponatinib, GVHD, and leukemia in the liver (n=1)

•Following sepsis syndrome, development of hypoxic hepatitis and cholestasis of infection (n=2) •Evidence was present for GVHD, hypoxic hepatitis, and cholestasis of infection (n=1)

^eInO-R arm: Of the 15 lymphoma patients classified as 'other hepatic disease', these were the clinical situations:

- Increased unconjugated serum bilirubin, Gilbert's Syndrome or hemolysis (n=2)
- Increased total serum bilirubin, months after InO-R exposure, cause unknown (n=2)
- Increased serum liver enzymes, no clear relation to InO-R exposure, cause unknown (n=11)

^fControl arm: Of the 4 lymphoma patients classified as 'other hepatic disease', these were the clinical situations:

- Increased unconjugated serum bilirubin, Gilbert's Syndrome or hemolysis (n=1)
- Increased serum liver enzymes, no clear associations, cause unknown (n=2)
- Ascites, cause unclear, tumor or pre-existing liver disease (n=1)

^gInO arm: For the ALL patient classified as 'not enough information', this was the clinical situation:

- Mild increase in total serum bilirubin (mostly unconjugated) in InO cycles 1, 4 – 6; other liver tests normal; no further evaluation at study site

^hInO-R arm: Of the 2 lymphoma patients classified as 'not enough information', these were the clinical situations:

- Delayed increase in alkaline phosphatase, GGT, and bilirubin after InO-R exposure, not investigated at study site (n=1)

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Increased total serum bilirubin after febrile neutropenia, not investigated at study site (n=1)

Control arm: Of the 2 lymphoma patients classified as 'not enough information', these were the clinical situations:

- Ascites detected before and after therapy, cause unknown (lymphoma or pre-existing liver disease) (n=1)
- One day with mildly increased serum AST/ALT, not repeated (n=1)

Hepatic Events Adjudication Board classifications of liver disorders in ALL patients treated on Study B1931022, after the start of conditioning therapy in preparation for hematopoietic cell transplantation.

Table 3.

HE/AB classification	InO, followed by HCT (n=79)	Standard chemotherapy ^a , followed by HCT (n=34)	P-value (Fisher's exact test)
Liver disorder reported after the start of conditioning therapy and HCT			
YES	27 (34%)	4 (12%)	0.0203
NO	52 (66%)	30 (88%)	
SOS-all degrees of certainty, all degrees of severity	21 (27%)	3 (9%)	0.0442
SOS-definite, all degrees of severity			
Mild	0	0	–
Moderate	3	1	–
Severe	4	0	–
SOS-probable, all degrees of severity			–
Mild	0	0	–
Moderate	4	0	–
Severe	4	1	–
SOS-possible, all degrees of severity			
Mild	2	0	–
Moderate	3	1	–
Severe	1	0	–
SOS, moderate + severe, all degrees of certainty	19 (24%)	3 (9%)	0.0726
Nodular regenerative hyperplasia	1 (1.3%)	0	–

HEA/B classification	InO, followed by HCT (n=79)	Standard chemotherapy ^a , followed by HCT (n=34)	P-value (Fisher's exact test)
Other hepatic disease	1 (1.3%) ^b	0	--
Intrahepatic cholestasis	3 (3.8%)	1 (3%)	

^aFludarabine/cytarabine/granulocyte colony-stimulating factor (FLAG), cytarabine / mitoxantrone, or high-dose cytarabine.

^bThis patient, classified as 'other hepatic disease' in the InO arm, had evidence of acute GVHD (including liver involvement) plus the effects of infection on hepatocyte transport of bilirubin, plus possible tumor cell infiltration of the liver.

Table 4.

Univariate analysis of risk factors for SOS in patients who underwent allogeneic hematopoietic cell transplantation following treatment for ALL on Study B1931022.

Risk factors for development of post-HCT Sinusoidal Obstruction Syndrome	Patients who developed SOS after HCT (n=24)	Patients who did not develop SOS after HCT (n=89)	P-value^a
Patient demographic factors			
Age in years (median, range)	41 (23–67)	37 (19–71)	0.0435
Female gender (n, %)	13 (54%)	42 (47%)	0.6471
White race (n, %)	14 (58%)	71 (80%)	0.0592
Asian race (n, %)	7 (29%)	11 (12%)	0.0605
Non-white, non-Asian race (n, %)	3 (13%)	7 (8%)	0.4404
Medical history before randomization and treatment on protocol			
Time (months) from diagnosis of ALL to randomization (median, range)	11.8 (2.7–64.9)	15.6 (1.3–94.2)	0.3596
Exposure to 6-mercaptopurine therapy (n, %)	10 (42%)	43 (48%)	0.6478
Exposure to 6-thioguanine therapy (n, %)	0	12 (14%)	0.0672
Exposure to methotrexate therapy (n, %)	21 (88%)	79 (89%)	1.0
Prior treatment with HCT (n, %)	8 (33%)	10 (11%)	0.0230
History of liver disease (n, %)	11 (46%)	16 (18%)	0.0073
Elevation of serum ALT, AST, or total serum bilirubin before randomization (n, %)	5 (21%)	20 (23%)	1.0
Factors from randomization to start of conditioning therapy for HCT			
Exposure to inotuzumab (n, %)	21 (88%)	58 (65%)	0.0442
Time in days from last dose of study therapy (median, range)	32.5 (9–168)	40.0 (4–404)	0.5888
Any liver disorder during study period, as adjudicated (n, %)	3 (13%)	6 (7%)	0.3975
DILI during study period, as adjudicated (n, %)	2 (8%)	2 (2%)	0.1975
Cholestatic disease during study period, as adjudicated (n, %)	1 (4%)	3 (3%)	1.0
Peak total serum bilirubin (mg/dL) during study period (median, range)	0.8 (0.3–2.6)	0.8 (0.2–10.1)	0.2240
Peak serum ALT (U/L) during study period (median, range)	37 (16–171)	43 (17–248)	0.1602
Peak serum alkaline phosphatase (U/L) during the study period (median, range)	172 (66–765)	111 (52–617)	0.0004
New induction therapy for ALL following study period (n, %)	5 (21%)	19 (21%)	1.0
Total serum bilirubin (mg/dL) before start of conditioning therapy (median, range)	0.6 (0.3–2.3)	0.5 (0.1–2.3)	0.0628

Risk factors for development of post-HCT Sinusoidal Obstruction Syndrome	Patients who developed SOS after HCT (n=24)	Patients who did not develop SOS after HCT (n=89)	P-value^a
Serum ALT (U/L) before start of conditioning therapy (median, range)	22 (9–97)	30 (6–183)	0.1775
Serum alkaline phosphatase (U/L) before start of conditioning therapy (median, range)	158 (58–741)	97 (50–580)	0.0001
WBC ($\times 10^9/L$) before start of conditioning therapy (mean $\pm$ SD, median, range)	4.5 $\pm$ 6.5, 3.0 (0.1–33.7)	5.1 $\pm$ 4.3, 4.2 (0.2–26.3)	0.0439
Peripheral blast count ($\times 10^9/L$) before start of conditioning therapy (mean $\pm$ SD, median, range)	1357 $\pm$ 5328, 0.0 (0–25275)	42.2 $\pm$ 168.9, 0.0 (0–1035)	0.0827
Thrombocytopenia (platelet count $<100 \times 10^9/L$) before start of conditioning therapy (n, percent)	19 (79%)	34 (38%)	0.0004
Factors related to allogeneic HCT procedure			
Myeloablative conditioning regimen (n, %)	14/22 (64%)	59/86 (69%)	0.7990
Cyclophosphamide-containing myeloablative regimen (n, %)	6 (25%)	36 (40%)	0.2342
Busulfan-containing myeloablative regimen(n, %)	5/23 (22%)	11/87 (13%)	0.3194
Melphalan-containing myeloablative regimen (n, %)	2 (8%)	2 (2%)	0.1975
ThioTEPA-containing myeloablative regimen (n, %)	3 (13%)	4/88 (5%)	0.1673
Total body irradiation (12 Gy)-containing myeloablative regimen (n, %)	4 (17%)	34 (38%)	0.0542
Unrelated donor (n, %)	18 (75%)	51/86 (59%)	0.2325
HLA-matched donor (n, %)	15 (63%)	64/86 (74%)	0.3061
GVHD prophylaxis with calcineurin inhibitor and methotrexate (n, %)	12 (50%)	27 (30%)	0.0915
GVHD prophylaxis containing sirolimus (n, %)	0	3 (3%)	1.0
Post-HCT cyclophosphamide GVHD prophylaxis (n, %)	1 (4%)	4 (5%)	1.0

^aTwo-sided p-value calculated by Fisher's exact test for categorical variables and the Wilcoxon Rank-sum test for continuous variables.

Table 5.
Multivariate risk factor analysis for SOS following conditioning therapy and allogeneic HCT among ALL patients enrolled on Study B1931022 (N = 111).

Two patients with missing data were excluded from the analysis.

Risk Factor	Odds Ratio	95% Confidence Interval	P-value
Thrombocytopenia (platelet count $<100 \times 10^9/L$) before start of conditioning therapy	4.223	1.279 – 13.939	0.018
History of liver disease before study enrollment	3.498	1.065 – 11.492	0.039
HCT before study enrollment	3.180	0.804 – 12.572	0.099
GVHD prophylaxis with a calcineurin inhibitor and methotrexate	3.133	0.995 – 9.866	0.051
Serum alkaline phosphatase (U/L) before conditioning therapy	1.066	1.000 – 1.011	0.050

Table 6. Study B1931022 subjects with ALL whose Investigator diagnoses were discordant from HEAB diagnoses.

Study Arm	Investigator Verbatim Term	HEAB Diagnosis	HEAB Summary
Discordant diagnoses—after randomization, before HCT			
InO	VOID	Intrahepatic cholestasis	Polymicrobial sepsis, followed by multiorgan failure, brain abscess, and jaundice.
InO	VOID	Intrahepatic cholestasis	Polymicrobial sepsis followed by jaundice.
Discordant diagnoses—after HCT			
InO	VOID	Non-alcoholic steatohepatitis and cholestasis	Metabolic syndrome and obesity at baseline. During study therapy, jaundice following infection; liver biopsy showed cholestasis and changes typical of non-alcoholic steatohepatitis.
InO	Hepatotoxicity	SOS, probable, severe and intrahepatic cholestasis	History of prior myeloablative HCT; then InO exposure, then a second myeloablative HCT, complicated by polymicrobial sepsis, jaundice, and ascites.
InO	Multi-system dysfunction	SOS, probable, moderate and intrahepatic cholestasis	History of prior myeloablative HCT and InO exposure, then a second myeloablative HCT; complicated by severe viral infection, jaundice, ascites, renal insufficiency, and GVHD.