Interactive Safety Graphic – Hepatic Safety Explorer

User's Manual

for

Version 1.1

A product of the DIA-ASA Biopharm Working Group

Interactive Safety Graphic – Hepatic Safety Explorer

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Introduction

Early specific indicators of drug-induced hepatic injury include elevations of hepatic transaminases and total bilirubin. However, the diagnosis of drug-induced liver injury (DILI) is one of exclusion, having eliminated other possible causes of the laboratory and clinical abnormalities. As first proposed by Dr. Hyman Zimmerman (1978) based on clinical presentation, and subsequently refined by FDA as an evaluation of biomarkers, the concept of "Hy's Law" became adopted as a means to identify instances indicative of the potential for DILI. The predictive value of Hy's Law has been validated by studies in Sweden (Bjornsson & Olsson 2005) and Spain (Andrade et al. 2005). Dr. Ted Guo, a statistician at FDA, and Dr. John Senior were the first to develop a graphical tool to screen laboratory datasets for elevations of transaminases and bilirubin that met the definition of possible Hy's Law cases; the application was called eDISH for evaluation of drug-induced serious hepatotoxicity (Senior 2014). This approach to the graphical display of hepatic laboratory data has subsequently been adopted by safety specialists in industry and academia.

The DIA-ASA Biopharm Working Group is interested in developing new interactive tools that expand upon the static nature of existing graphics, such as eDISH. Beyond just a tool for signal detection, the interactive tool also provides data exploration capabilities to facilitate signal evaluation. This interactive safety graphic builds upon the static eDISH graph to afford customization of the analysis and the ability to explore cases that appear in each of the quadrants of interest: potential Hy's Law cases, Temple's Corollary cases and isolated hyperbilirubinemia cases. For each such case of interest, the underlying data can be evaluated for evidence supporting or discounting a contributory role by the drug of interest. This user's manual provides not only instructions concerning the features of this tool, but also a suggested workflow for evaluating the characteristics of any cases meeting the conditions for a potential Hy's Law case, a case of Temple's Corollary or a case of hyperbilirubinemia. Each of the suggested evaluation steps is accompanied by information supported by the medical literature concerning how to interpret the findings of each evaluation. The user is also referred to the FDA's guidance document for a review of their approach to evaluating signals of potential DILI (FDA 2009).

It is important to understand that this tool is intended only to facilitate *exploration* of the data and to provide an initial evaluation of potential causes of the findings. The user should be cautioned that this tool is **not intended** to provide sufficient evidence to support a conclusion of drug-induced liver injury. DILI is a diagnosis of exclusion which requires additional evaluations beyond the scope of this tool. For example, serology is important to rule out the possible role of hepatitis infection, which is not included in this tool. A comprehensive discussion of additional tests to consider outside the scope of this tool in the evaluation of possible cases of DILI is provided by Chalasani et al. (2014). As stated by Dr. John Senior, one of the originators of FDA's eDISH graphic, "DILI cannot be diagnosed by serum chemistries alone, nor by liver biopsies, but requires pertinent clinical information" (Senior 2014). This tool is also

not presently validated. Before making conclusions regarding product risk, findings derived from this tool should be confirmed via validated methods in keeping with the user's organization's SOPs.

For instructions on how to how to download and install R Shiny on your computer, please refer to: https://jennybc.github.io/2014-05-12-ubc/r-setup.html. For instruction on how to start the Shiny app, please refer to: https://github.com/ASA-DIA-InteractiveSafetyGraphics/safetyGraphics/wiki/Vignette:-Shiny-User-Guide.

An Introduction to eDISH

The eDISH (evaluation of drug-induced serious hepatotoxicity) graph was conceptualized and created by Dr.s Ted Guo and John Senior at FDA (Senior 2014) in order to evaluate laboratory chemistry data for instances meeting the criteria for "Hy's Law" cases that might predict the risk for significant drug-induced hepatocellular injury. The program looked for indicators of hepatocellular injury by serum alanine aminotransferase (ALT) elevation and of whole liver dysfunction by total bilirubin elevation. For conservative purposes, to preserve sensitivity of detecting nearly all cases, low cut-off levels were employed: 3xULN for ALT and 2xULN for total bilirubin.

The cut-off thresholds produce four quadrants: a lower left quadrant that contains most patients with normal or near normal peak values for both variables; an upper left quadrant that contains those with elevated bilirubin levels but not significant ALT elevation; a lower right quadrant showing those with elevated ALT but not total bilirubin, indicating hepatocellular injury without whole liver dysfunction; and patients in the right upper quadrant who showed clinically significant hepatocellular injury and whole liver dysfunction by elevations of both ALT and total bilirubin, not necessarily on the same test day. The upper right quadrant represents possible Hy's Law cases, based on Dr. Hyman Zimmerman's observation that drug-induced hepatocellular injury (marked elevation of serum transaminases with no or minimal elevations in serum alkaline phosphatase) accompanied by clinical jaundice is associated with a mortality of at least 10% (range 5–50%) (FDA 2009, Kaplowitz 2009). For the purpose of the graph, clinical jaundice was represented by a total bilirubin >2xULN. The right lower quadrant is labelled 'Temple's Corollary', reflecting an observation first made by Dr Robert Temple of the FDA that an isolated elevation of ALT may represent serious liver injury, even when examination of the Hy's Law quadrant was unrevealing and may indicate cases that could progress into the Hy's Law quadrant (FDA 2009). Cut-off levels were not established by data analyses but were based on expert opinions at a Fogarty International Conference in 1978 (Davidson et al. 1979) that concluded that ALT values >3xULN and total bilirubin >2xULN were ''markedly abnormal.''

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It is important to appreciate that the FDA guidance recognizes that not all subjects experiencing serum ALT >3x ULN and serum total bilirubin >2x ULN represent a serious liver safety signal. A Hy's Law case requires fulfilling the following criteria (FDA 2009):

1) A higher incidence of 3-fold or greater elevations above the ULN of ALT or AST than the (non-hepatotoxic) control drug or placebo
2) Patients with ALT/AST elevations demonstrate elevation of serum total bilirubin to >2xULN, without initial findings of cholestasis (elevated serum alkaline phosphatase, generally considered to be >2xULN)
3) No other reason can be found to explain the combination of increased ALT/AST and total bilirubin, such as viral hepatitis A, B, or C; preexisting or acute liver disease; or another drug capable of causing the observed injury.

Thus, to be entirely accurate, points that appear in the upper right quadrant of a graph of ALT (or AST) versus total bilirubin should only be considered as "potential" Hy's Law cases until further evaluation is performed.

Short Cut: For an abbreviated workflow that focuses on the specific features of a potential Hy's Law case, please refer to Appendix 4.

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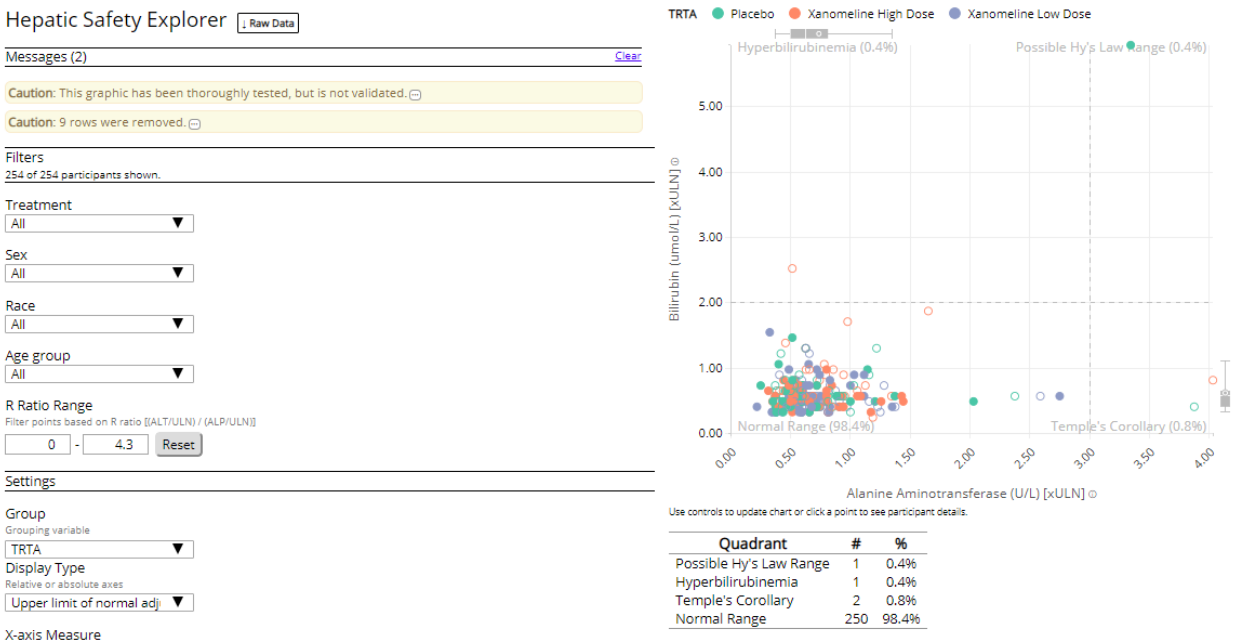
Hepatic Safety Explorer Graphic Features



Messages

When the interactive graphic is launched the system will display relevant messages. The first message is a standard caution that this tool has not been formally validated; as such it is intended for exploratory purposes only. Decisions based upon these analyses to define product risks should be founded on replicated analyses using the sponsor's internal validated tools and standard operating procedures.

Additional messages can include cautions concerning the dataset in use. Details of each of these can be accessed by clicking on the icon.

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Filters

When a filter is applied the graph indicates how many of the total population of patients is represented in the display.

For example: 249 of 254 partiticpants shown.

Treatment:

Depending on the what treatments are defined in the dataset, the display can be filtered to include only one of the available treatment arms. When “All” is selected, there is no filtering based on treatment assignment.

Sex:

The data can be filtered to display only female (F) or male (M) patients. When “All” is selected, there is no filtering based on sex.

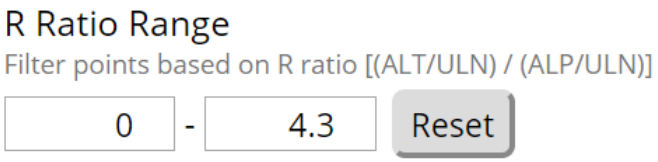
Race:

Depending on how race is defined in the dataset, the display can be filtered to include only one of the available racial types. When “All” is selected, there is no filtering based on race.

Age Group:

The data can be filtered based on age groups, depending on the availability of age ranges in the dataset; for example, <65 years, 65-80 years and >80 years. When “All” is selected, there is no filtering based on age group.

R Ratio Range (ALT/ULN)/(ALP/ULN):

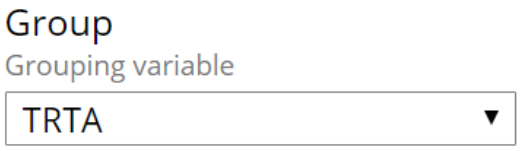


The R Ratio is a calculation to evaluate the extent to which ALT elevations may be related to a cholestatic process versus a hepatocellular one. The graph can display cases where the R Ratio falls within a prespecified range. Refer to the clinical workflow for advice on the choice of values to define the range.

Reset Chart: After an adjustment is made on any of the above parameters, the user can return to the default state by clicking on the reset button: 

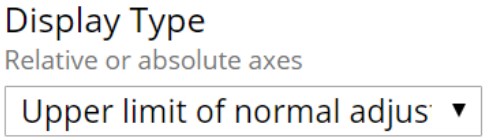
Settings

Group:



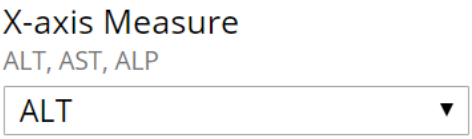
The data can be displayed by colors denoting the groupings chosen when previously configuring the graph. For example, Treatment (TRTA), Race (RACE) and Age Group (AGEGP1). If None is chosen, the subjects are not grouped by any parameter and all points have the same color.

Display Type:



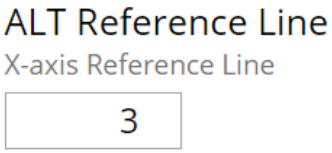
The display type defines how the X and Y axes are presented in terms of fold change from a reference point. The standard eDISH display is to present the transaminase and bilirubin data as fold change from the upper limit of normal for each respective laboratory value. The alternative mDISH approach is to display the laboratory data as the fold change from the patient's baseline for that variable. The default baseline for each laboratory value is based on DY == 0, but the user can specify the field to define the baseline value during the configuration. Refer to the clinical workflow for circumstances to use the mDISH display.

X-axis Measure:

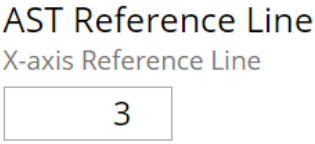


The X-axis can display ALT, AST or alkaline phosphatase individually. The current version of this tool displays either ALT or AST on the axis but presently cannot display both. Since the Hy's Law evaluation considers elevations of both ALT and/or AST, the user will need to change the X-axis to interrogate both transaminases. Alkaline phosphatase is included as a choice to further explore the data.

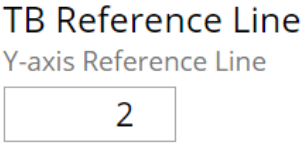
ALT Reference Line:



The ALT reference line (X-axis reference line) defaults to 3, the standard threshold for the graph. However, there are certain circumstances in which the user may wish to change the threshold. This can be accomplished by either entering a new value into the box or, alternatively, by using the mouse to drag the line on the graph to the desire value. Refer to the clinical workflow for examples of when to consider adjusting the ALT threshold. Note: if the X-axis Measure is set to AST, the reference line variable changes to AST:

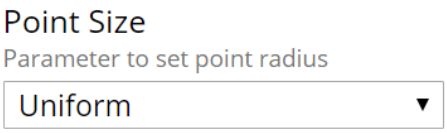


TB Reference Line:



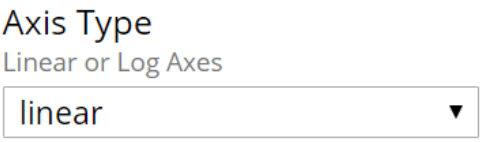
The total bilirubin reference line (Y-axis reference line) defaults to 2, the standard threshold for the graph. However, there are certain circumstances in which the user may wish to change the threshold. This can be accomplished by either entering a new value into the box or, alternatively, by using the mouse to drag the line on the graph to the desire value.

Point Size:



The size of the points displayed in the graph can be changed from the default of a uniform point size to one based on the value of 4 other laboratory values: ALT, AST, alkaline phosphatase and total bilirubin. The larger the value of the variable, the larger the point size. This can provide additional quantitative data to the graph. For example, while displaying ALT on the X-axis, setting the point size based on the AST value can provide useful information as to the extent to which AST elevations coincide with ALT elevations. Similarly, if the point size is based on the alkaline phosphatase variable, the user can have a sense of the extent to which elevated ALT/ total bilirubin values may be associated with evidence of cholestasis.

Axis Type:



The format of the X and Y axes can be changed from the default of a linear axis to a log display. Using a log axis can sometimes be useful to better identify far outliers.

Highlight Points Based on Timing:

The time interval between peak transaminase and total bilirubin values is an important aspect in the evaluation process, as will be described in more detail in the clinical workflow. The user can identify cases where the peak values occur with a predefined time period. In this case the default is 30 days; that is, the case is displayed as a filled circle ● when the peak transaminase and peak total bilirubin values occur within 30 days. The default value is defined when the chart is first configured. A shorter time period can be utilized, such as 14 days, in which only cases with peak values within 14 days will be displayed as filled circles. A time window of any amount can be entered into this field. Cases in which the peak values occur outside this time window are displayed as open circles ○. (Note: the default time window is defined during the configuration step.)

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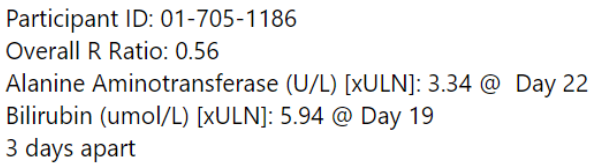
Features

Hover the cursor over a point on the graph and a window opens displaying basic information about the patient: participant ID, the overall R Ratio, ALT value of fold change (from either ULN or baseline depending on Display Type choice), study day of ALT value, total bilirubin fold change (from either ULN or baseline depending on Display Type choice), study day of total bilirubin value, how many days apart are the peak ALT and peak total bilirubin values.



If AST is chosen as the x-axis, the fold change and study day of the value will represent the AST data.

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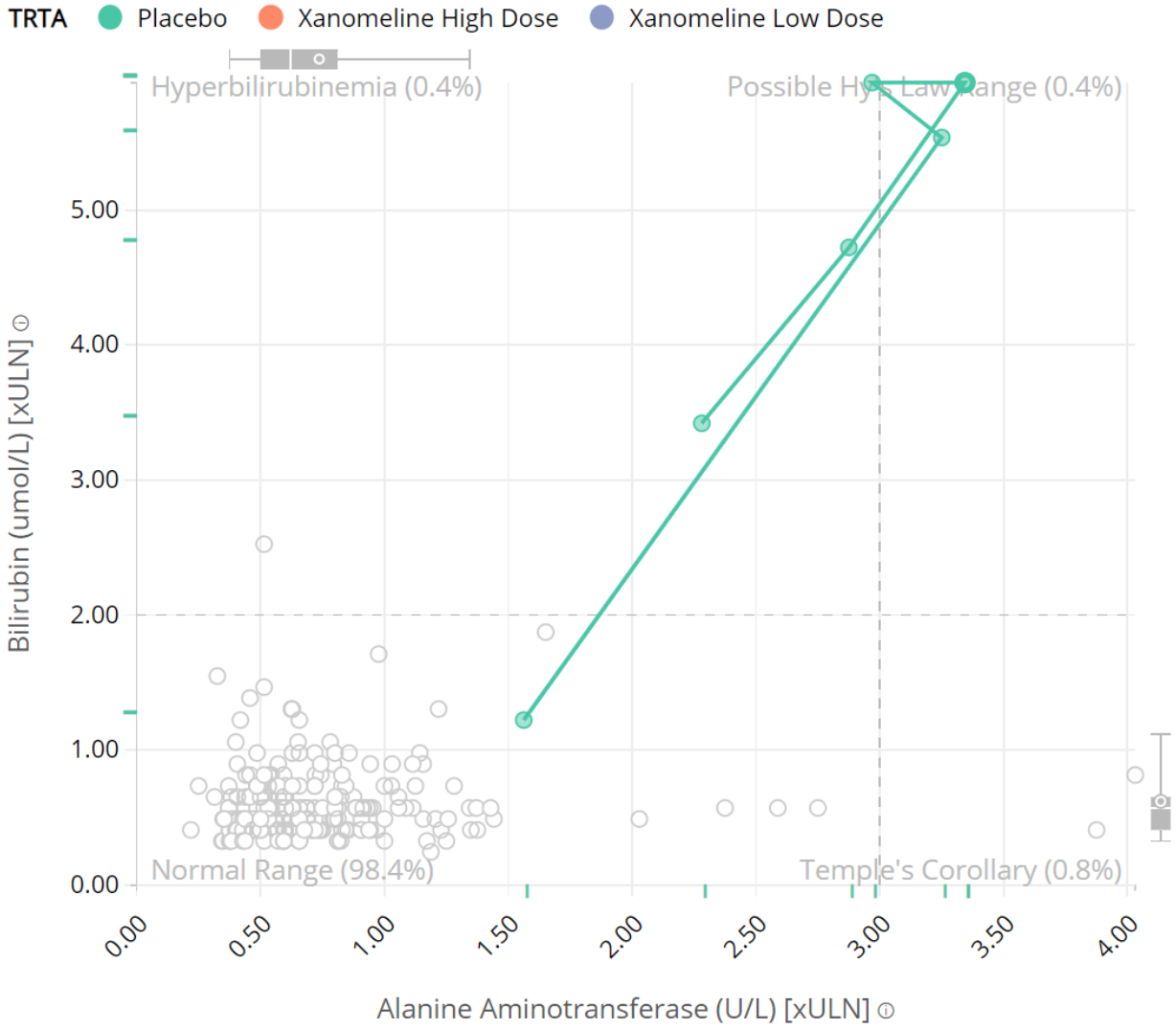
Click on a point on the graph to launch several features.

1) An animated graph is displayed showing the trajectory of that patient's ALT (or AST) and corresponding total bilirubin values over time.



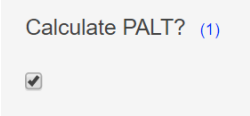
2) Participant demographic details are displayed below the graph.

Participant Details

Subject Identifier	Treatment	ALT Elevated?	RACE	AGEGR1	R Ratio	P_{ALT}
01-705-1186	Placebo	Yes	WHITE	>80	0.59	1.40

Note that these details include the calculated value of P_{ALT}, a measure of hepatocyte loss. The interpretation of the P_{ALT} value is described in further detail in the clinical workflow sections on Hy's Law and Temple's Corollary.

Please note that in order for the P_{ALT} value to be calculated, the checkbox in the settings page must be checked.



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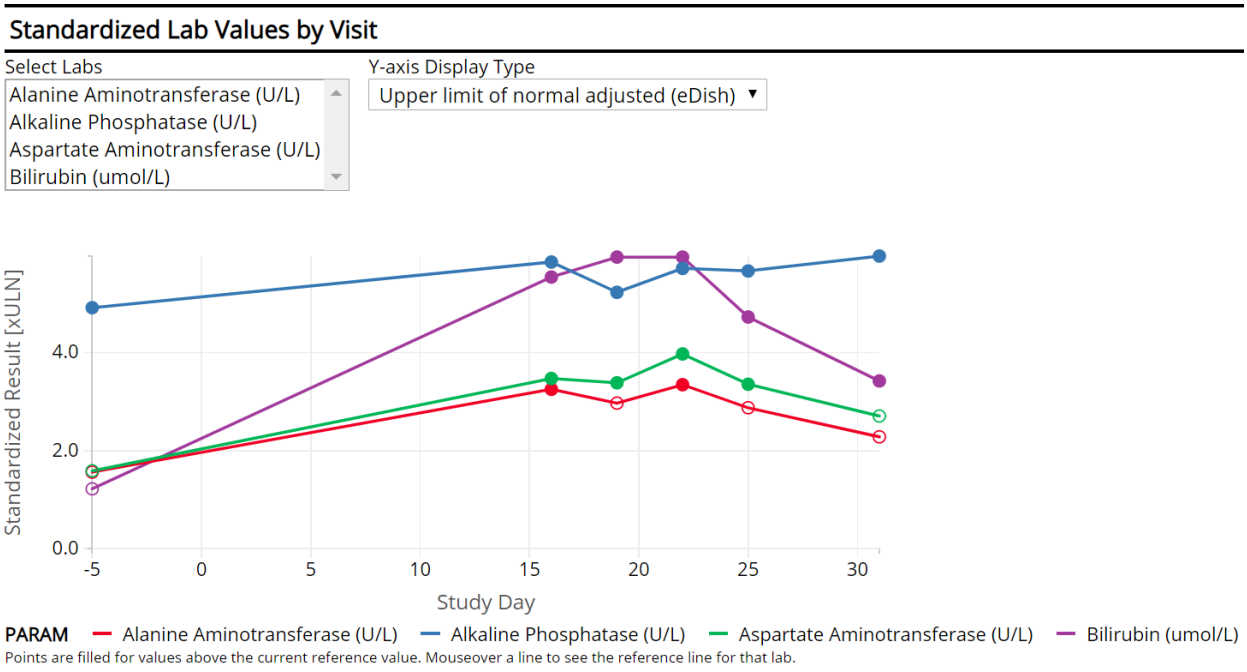
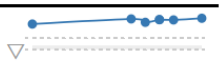
3) Standardized Lab Values by Visit are displayed in a graph of fold change of ALT, AST, total bilirubin and alkaline phosphatase over time. By default, all 4 analytes are displayed concurrently. Single analytes can be displayed by clicking on the analyte of interest in the Select Labs window. The fold change from either ULN (eDISH) or from baseline value (mDISH) can also be chosen in this display via the Y-axis Display Type window.



4) The Raw Lab Values Summary Table by default displays individual graphs of ALT, AST, total bilirubin and alkaline phosphatase over time in a table listing the number of measurements, the minimum, median and maximum values.

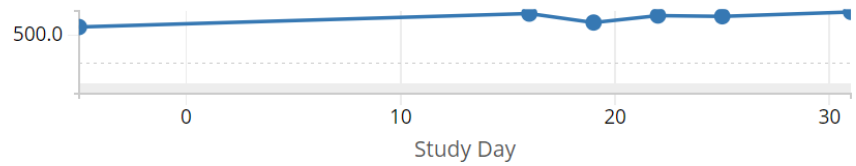
Raw Lab Values Summary Table

Show 1 additional measure: ☐

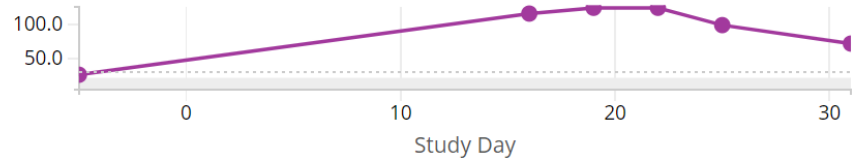
Measure	N	Min	Median	Max	
Alkaline Phosphatase (U/L)	7	565	657	686	
Bilirubin (umol/L)	7	25.65	116.28	124.83	
Aspartate Aminotransferase (U/L)	7	54	115	135	
Alanine Aminotransferase (U/L)	7	50	95	107	

Clicking on the down arrow to the left of the graph expands the graph and includes a greyed-out area representing the normal range for that analyte.

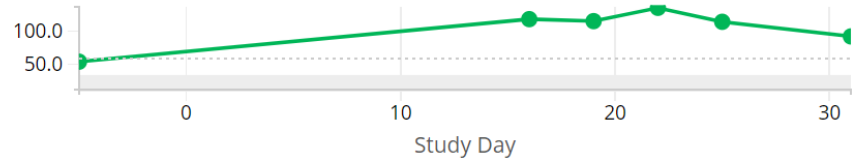
Measure	N	Min	Median	Max	
Alkaline Phosphatase (U/L)	7	565	657	686	△ Minimize Chart



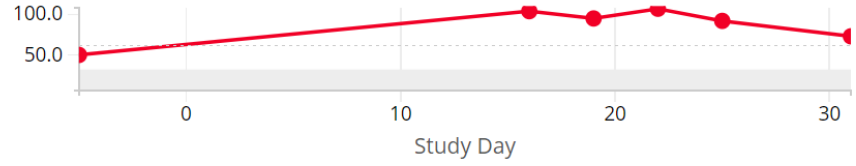
Bilirubin (umol/L)	7	25.65	116.28	124.83	△ Minimize Chart



Aspartate Aminotransferase (U/L)	7	54	115	135	△ Minimize Chart



Alanine Aminotransferase (U/L)	7	50	95	107	△ Minimize Chart



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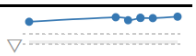
In addition to these 4 default analytes, all additional analytes represented in the dataset can be displayed by clicking the Show X Additional Measures, where X is the number of additional analytes detected by the app. For example, availability of direct (conjugated) and indirect (unconjugated) bilirubin is useful when evaluating instances of hyperbilirubinemia. When these analytes are included in the uploaded dataset they will be available to the user when the Show X Additional Measures box is checked. In this example, potassium levels were also in the dataset and are now displayed. But any laboratory analyte in the dataset can be displayed.

Measure	N	Min	Median	Max	
Alkaline Phosphatase (U/L)	7	565	657	686	
Bilirubin (umol/L)	7	25.65	116.28	124.83	
Aspartate Aminotransferase (U/L)	7	54	115	135	
Alanine Aminotransferase (U/L)	7	50	95	107	
Potassium (mmol/L)	6	3.5	3.65	4.1	

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Hepatotoxicity Evaluation Workflow

The diagnosis of drug-induced liver injury is one of exclusion, where it is important to first identify possible confounding factors giving rise to elevations in transaminase and total bilirubin levels before concluding that exposure to the drug of interest has resulted in hepatoxicity. A number of such evaluations can be conducted within the current version of the interactive graphic. The following flow diagrams illustrate a proposed method of working through important analyses that will gather data that supports or discounts a causal role for the drug of interest. At the conclusion of the workflow, and with the consideration of additional data elements, the user will be in a better position assess the extent to which the drug of interest contributed to the observed laboratory abnormalities. However, it is important to stress that additional evaluation of case details must be performed that is outside the current scope of this tool (e.g., serology) before it can be concluded that the drug of interest is the causative factor in a case of DILI.

The workflow consists of a number of decision steps and suggested evaluations. For each evaluation, a discussion of the rationale and means of interpreting the results is provided based on the medical literature and best practices.

The workflow is separated into sections accompanied by descriptions of the decision steps and suggested evaluations. A full depiction of the workflow steps for potential Hy's Law, Temple's Corollary and Hyperbilirubinemia cases are provided in Appendices 1, 2 and 3, respectively.

Short Cut: In order to facilitate a rapid review of hepatic laboratory data in order to distinguish which cases merit referral to a "hepatic board", a simple one-page evaluation guide is provided in Appendix 4.

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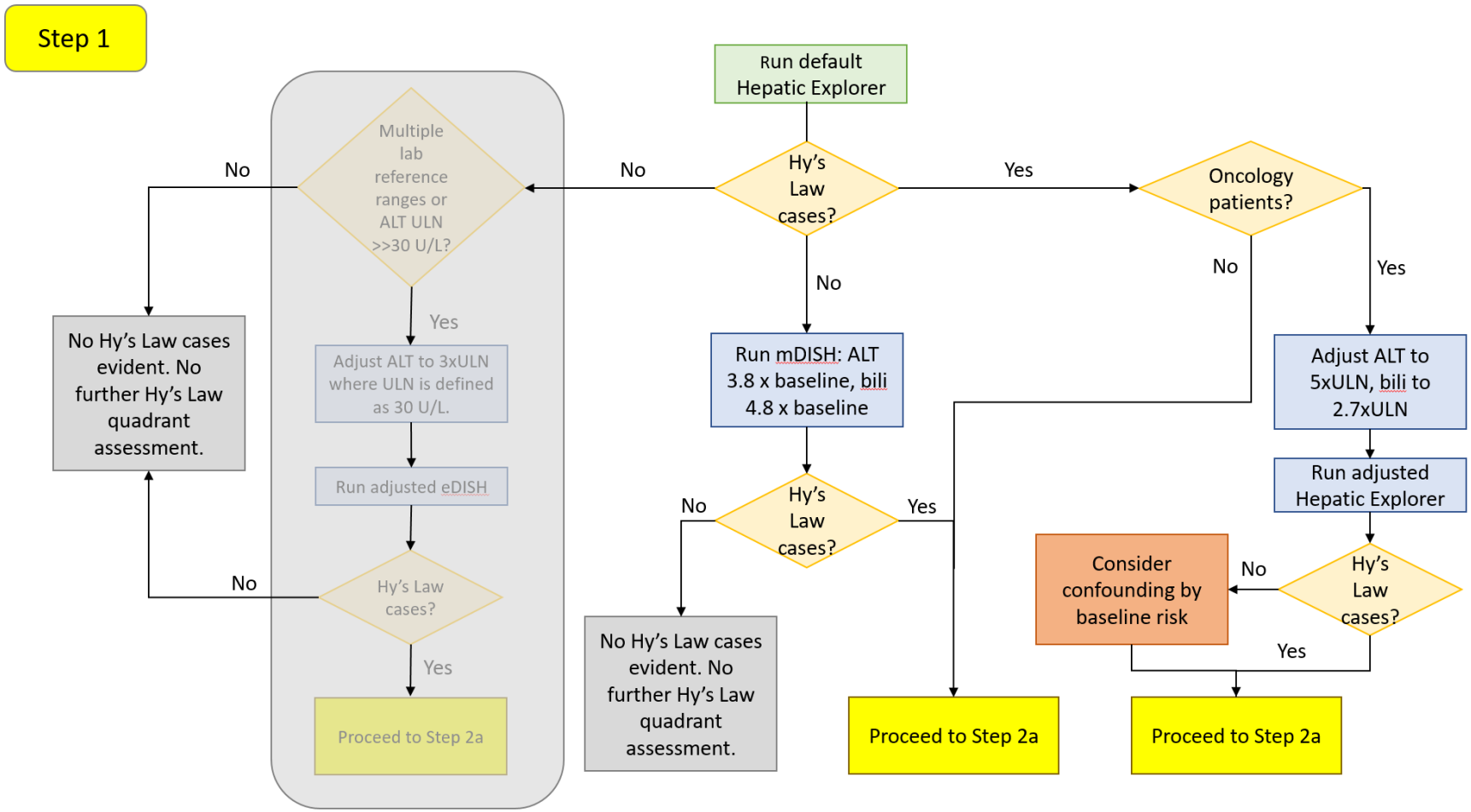


Note: The grayed-out portion is not available in Version 1.0 but is intended to be included in later version.

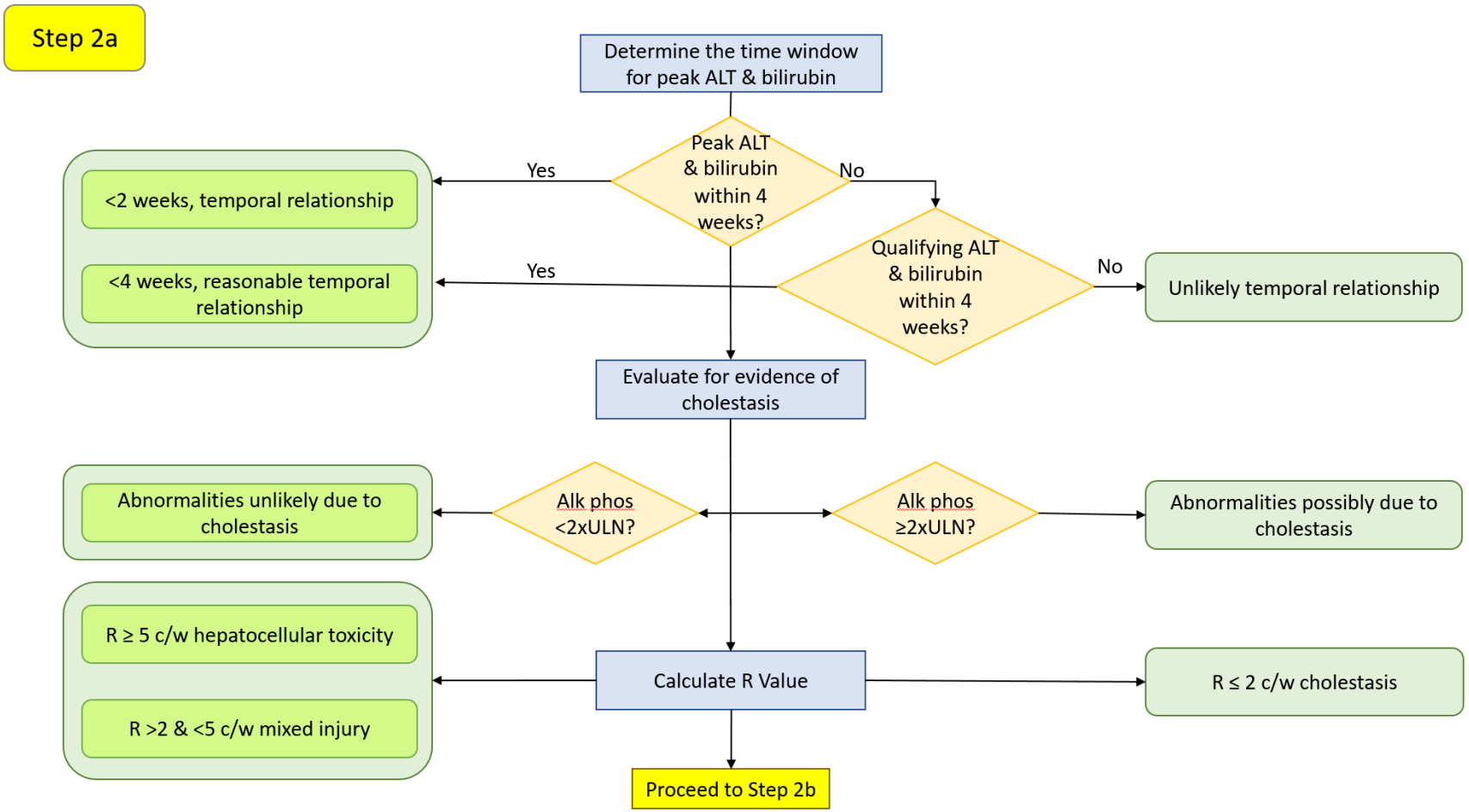
Hy's Law Quadrant Evaluation

Step 1

Hy's Law cases?	**No**: After loading the dataset of interest, allow the tool to plot the results using the default settings. If no cases appear in the upper right potential Hy's Law quadrant, run an mDISH analysis. Patients may have clinically important changes in transaminase and bilirubin levels that don't meet Hy's Law definition when evaluated using the default fold-change from the upper limit of normal, particularly if patients begin drug treatment with relatively low values. The alternative is to perform the analysis on the basis of fold-change from baseline. The baseline-corrected approach, called mDISH (modified DISH), would be more sensitive to drug effects and is more consistent across laboratories (Ozer et al. 2010, Lin et al. 2012). Baseline-corrected data may also be used in populations with previous liver injury and abnormal liver biochemistry prior study drug administration (Aithal et al. 2011). For a generally healthy study population, the boundary thresholds in mDISH are recommended to be 3.8 x baseline for ALT and 4.8 x baseline for total bilirubin (Lin et al. 2012). Adjust the ALT Reference Line and the TB Reference Line fields accordingly. Using a single value to establish a baseline is not optimal considering the within-subject variation in liver tests (Merz et al. 2014). A more suitable determination of baseline may consist of two measurements at least two weeks but not more than two months apart. This tool presently only allows you to specify a single "baseline" value in the settings. However, if the dataset contains more than one pre-dose value, e.g., screening and baseline, adjust the "baseline" value in Settings and re-run the mDISH analysis. If the mDISH analysis yields no potential Hy's Law cases, then further analyses are not necessary. (Note this will change when the fold change from a fixed ULN function will be implemented in a later version) If the mDISH analysis yields one or more potential Hy's Law cases, proceed to Step 2a.
	Yes: The appearance of cases in the potential Hy's Law quadrant could be the result of underlying risk factors in the population under study. Proceed to the next decision step: Oncology Patients?
Oncology patients?	**No**: Proceed to Step 2a.

	Yes: Oncology patients, particularly those with advanced disease, represent a population who often demonstrate elevated transaminase and bilirubin values at baseline due to extensive pretreatment and/or presence of liver metastases. Such patients may appear in the Hy's Law quadrant without necessarily experiencing drug-induced liver injury. A review of oncology patients, with and without evidence of liver metastases, recommended adjusting the ALT and total bilirubin thresholds (Parks et al. 2013). In patients without liver metastases, set the ALT threshold to 4.8 x ULN and bilirubin to 2.5 x ULN. In patients with liver metastases, set the ALT threshold to 5.5 x ULN and bilirubin to 3.0 x ULN. In patients either with or without known liver metastases, set the ALT threshold to 5.0 x ULN and bilirubin to 2.7 x ULN. Set these values in the ALT Reference Line and the TB Reference Line fields. If the adjusted thresholds result in the same potential Hy's Law cases, proceed to Step 2a. If the adjusted threshold result in the loss of cases from the potential Hy's Law quadrant, consider that their initial appearance as potential Hy's Law cases could have been the result of confounding by the underlying disease process. Proceed to Step 2a.
	Note: Other conditions may result in elevations of transaminases and/or bilirubin; e.g., right heart failure/hypotension, connective tissue disorders involving the liver, inflammatory bowel disease, non-alcoholic steatohepatitis, viral hepatitis and use of total parenteral nutrition (Ozer et al. 2010). However, recommendations for adjusted ALT and bilirubin thresholds are not available for these situations. In the case of the ischemic hepatitis that develops with right heart failure, the elevation in bilirubin is due to unconjugated bilirubin in 24-81% of cases (Dunn et al. 1973), illustrating the utility of bilirubin fractionation. The user should consult a hepatologist for consideration of adjusting ALT and total bilirubin thresholds when the dataset includes patients with these conditions.

Interactive Safety Graphic – Hepatic Safety Explorer

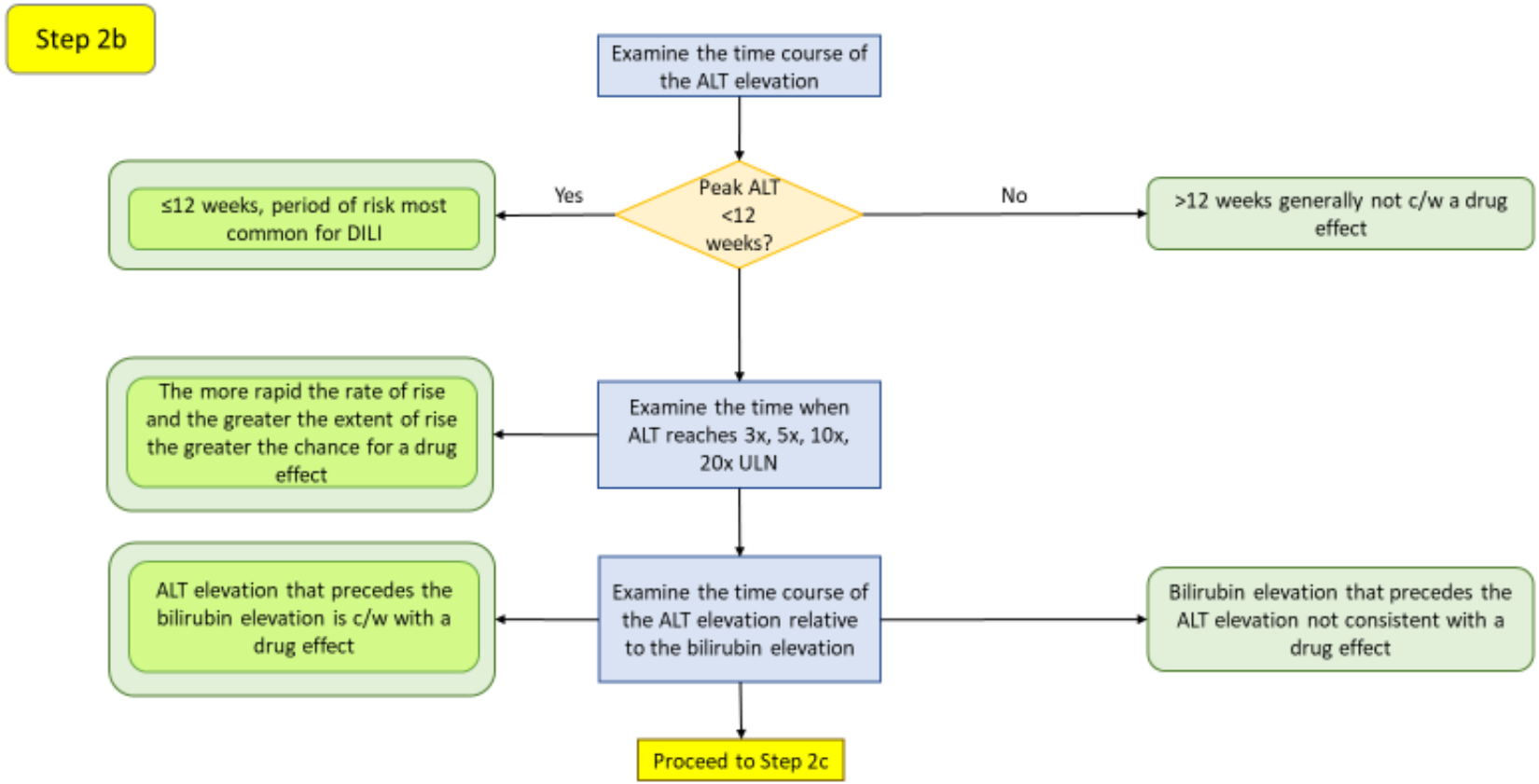


Step 2a

Peak ALT and bilirubin within 4 weeks?	**Yes**: Only elevations of bilirubin coincident with or shortly after peak ALT elevations are indicative of loss of hepatic function associated with liver injury (Merz et al. 2014, Longo et al. 2017). While there is no defined interval as a standard, peaks within 2 weeks are suggestive of DILI and up to 4 weeks may still indicate a drug effect. This can be assessed in the tool by defining the interval in the **Highlight Points Based on Timing** field**.** Alternatively, this can be manually reviewed by clicking on the point of interest in the Hy's Law quadrant and review the individual ALT and total bilirubin graphs displayed below the graph for that patient.
	No: Peak ALT and bilirubin values exceeding 4 weeks apart are less indicative of a drug-induced cause.
	Note: A peak total bilirubin level that precedes a peak ALT level is not a typical pattern for hepatocellular injury (Watkins et al. 2011).
Qualifying ALT and bilirubin within 4 weeks?	A limitation of the graph is the use of peak values to define the position of cases within the graph. "Qualifying" values are those that exceed the ULN threshold for either ALT or total bilirubin, but don't represent peak values (Merz et al. 2012). **Yes**: Qualifying ALT and total bilirubin values occurring within 2 weeks of each other, and with the rise in bilirubin following the transaminase rise, are suggestive of DILI. While there is no defined standard interval, qualifying values within 4 weeks may still indicated a drug-related effect. **No**: Qualifying ALT elevations exceeding 4 weeks apart are less indicative of a drug-induced cause.
Alk phos <2 x ULN?	**Yes**: A component of the Hy's Law definition is the presence of transaminase and bilirubin elevation without initial findings of cholestasis (e.g., elevated alkaline phosphatase) (FDA 2009). The lack of an elevated alkaline phosphatase has been defined as <2x ULN (Avigan 2010). Transaminase and bilirubin elevations meeting Hy's Law criteria in the absence of a concomitant elevation of alkaline phosphatase is indicative of hepatocellular injury. **No**: An elevated alkaline phosphatase level coincident with transaminase and bilirubin elevations may indicate a cholestatic source of the bilirubin elevation which could discount drug-related hepatocellular damage. However, this does not remove the possibility of drug-related cholestatic injury.

	Note: Alkaline phosphatase can be elevated by infiltrative diseases of the liver, tumors of hepatic and non-hepatic origin and bone diseases, including metastases to bone (AGA Clinical Practice Committee 2002). In such circumstances, these factors confound the ability to assess whether bilirubin elevations may be due to a cholestatic process.
Calculate R value	The R value (aka R ratio, R score) is calculated as [ALT/ULN]/[alkaline phosphatase/ULN] (Kullak-Ublick et al. 2017; Leise et al. 2014). R > 5 indicates hepatocellular injury R = 2-5 indicates mixed hepatocellular/cholestatic injury R < 2 indicates cholestatic injury A modified approach, called “new ratio” or nR, considers also the AST value in addition to the ALT value and uses whichever produced the highest fold change from the ULN (Robles-Diaz et al. 2014). Currently, this tool only calculates the R value based on ALT; calculation of the nR will need to be done manually at this time. Proceed to Step 2b.

Interactive Safety Graphic – Hepatic Safety Explorer

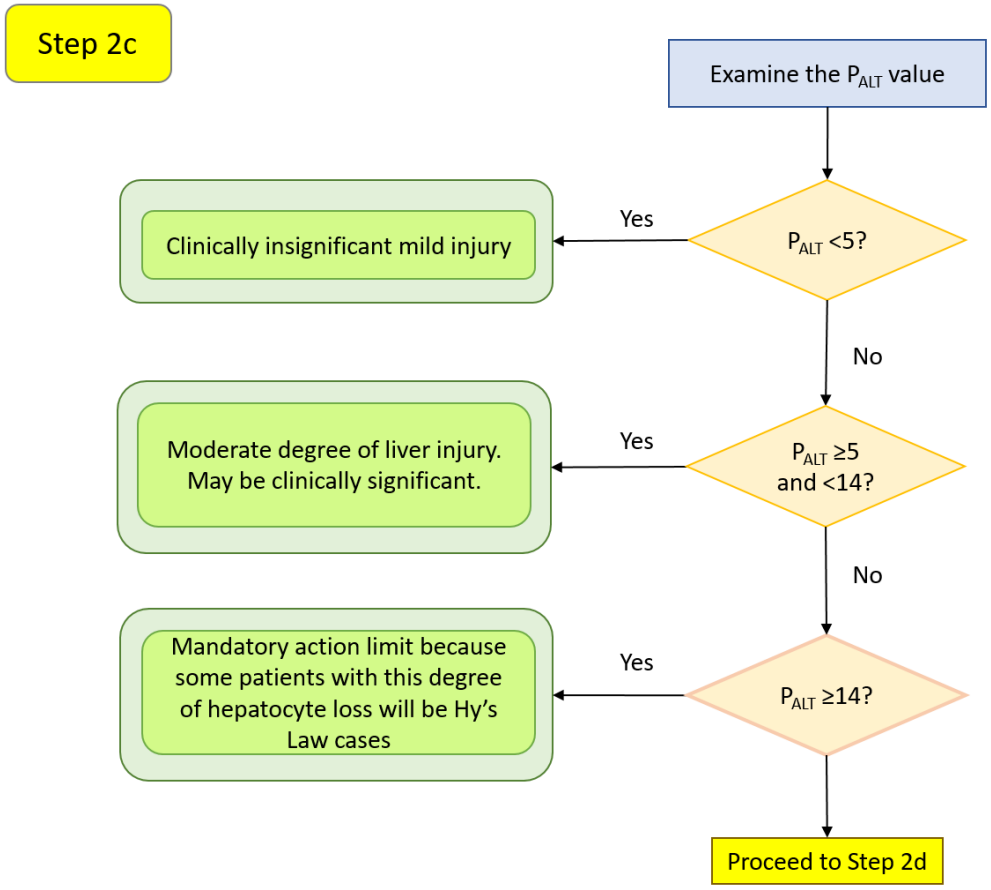


Interactive Safety Graphic – Hepatic Safety Explorer

Step 2b

Peak ALT <12 weeks?	**Yes**: When evaluating the time course of the ALT elevation, the first 12 weeks from drug initiation is generally the period of greatest risk of drug-induced hepatotoxicity (Hunt et al. 2007). Elevations within the first couple weeks often reflect adaptation to drug load rather than an actual hepatotoxic effect, particularly when the daily dose is several hundred milligrams and higher (Dara et al. 2016). The majority of patients who experience ALT elevations are not at risk of developing significant liver injury and will demonstrate resolution of the liver injury despite continued exposure to the drug as the liver develops immune tolerance or cellular adaptive responses (Dara et al. 2016). The ability to adapt appears to be a general phenomenon, while failure to adapt or defective adaptation leads to severe idiosyncratic injury. (Watkins 2005, Dara et al. 2016) Note that acute hepatobiliary obstruction or inflammation, such as caused by a gallstone, can result in an abrupt rise in transaminases, bilirubin and alkaline phosphatase (Green & Flamm 2002).
	No: Although instances of delayed hepatotoxicity have been reported, peak ALT levels in excess of 12 weeks are generally not consistent with a drug related effect.
Examine the time when ALT reaches 3x, 5x, 10x and 20x ULN.	Evaluating the time points at which various multiples of the ULN are reached (rate of rise) provides information on the acute nature of the reaction. The greater the rate of rise, the more acute the onset of the toxic effect suggestive of a drug-related effect.
Examine the time course of the ALT elevation relative to the bilirubin elevation	Only bilirubin elevations simultaneous with or soon after peak ALT elevations may indicate loss of hepatic function due to drug-induced liver injury. A time interval exceeding four weeks between both peaks may also speak against a causal correlation (Merz et al. 2014). Proceed to Step 2c.

Interactive Safety Graphic – Hepatic Safety Explorer

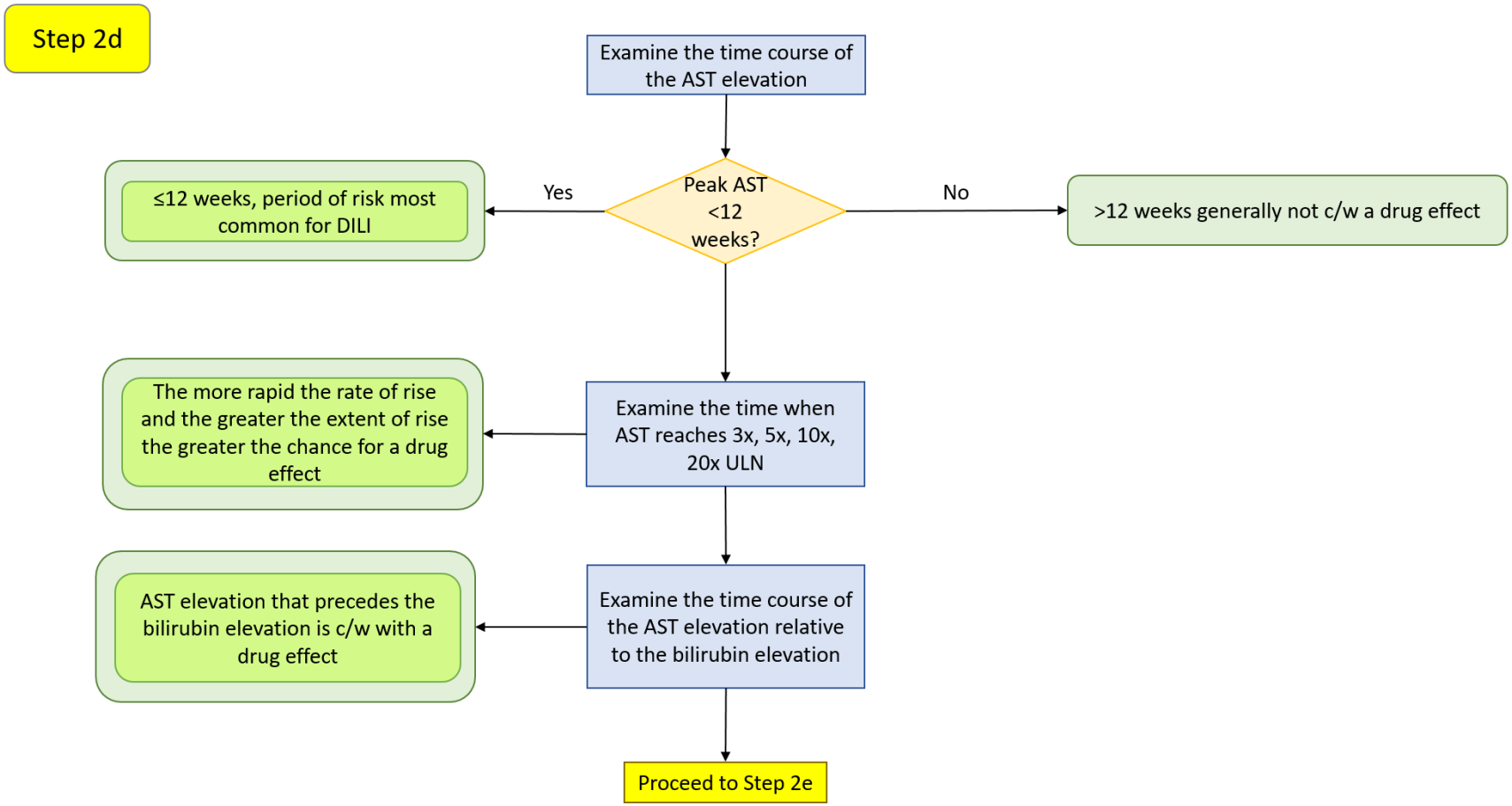


Interactive Safety Graphic – Hepatic Safety Explorer

Step 2c

P_{ALT} <5, ≥5 and <14 or ≥14?	P_{ALT}, calculated using an equation that uses peak ALT and the AUC of the serum ALT over time, provides an estimate of hepatocyte loss across a number of different time courses and patterns of injury (Chung et al. 2019). Based on acetaminophen overdose cases, it is reasonable to consider that 10% hepatocyte loss due to DILI as without clinical consequence and that >60% hepatocyte loss can be fatal. While multiple ALT values are preferable, it is possible to use P_{ALT} to estimate the maximum hepatocyte loss at any point during the course of elevated ALTs. **P_{ALT} values <5** represent clinically insignificant mild injury (95% upper CI limit of the amount of hepatocyte loss is <13%). **P_{ALT} values ≥5 and <14** represent a moderate degree of liver injury which may be clinically significant. Discontinue treatment if peak ALT >5x ULN for >2 weeks or ALT >3x ULN with fatigue, nausea, vomiting, right upper quadrant pain or tenderness, fever, rash, and/or eosinophilia (>5%) or INR >1.5. **P_{ALT} value of ≥14** (95% upper CI limit of the amount of hepatocyte loss is approaching 30%) may support liver injury sufficient to result in Hy's Law in some subjects (i.e., their serum bilirubin will exceed two times the ULN). **P_{ALT} values >30** are likely to lead to death (95% upper CI limit of the amount of hepatocyte loss is approaching 85%). When serum ALT elevations are observed in clinical trials, a common practice has been to search for correlations between drug exposure and the observed peak serum ALT values and thereby estimate safe exposure levels and hence optimal dosing schedules. Estimates of hepatocyte loss may be a better variable than peak serum ALT for this purpose. If an excellent correlation between drug exposure and hepatocyte loss is not evident, it is possible that interindividual differences in the underlying toxic mechanism(s) could be involved. This can now be addressed by incorporating quantitative systems toxicology models, such as DILIsym. Note that P_{ALT} was developed using models based on healthy livers. The estimate may not accurately predict hepatocyte loss in children and patients with preexisting liver diseases. Proceed to Step 2d.

Interactive Safety Graphic – Hepatic Safety Explorer



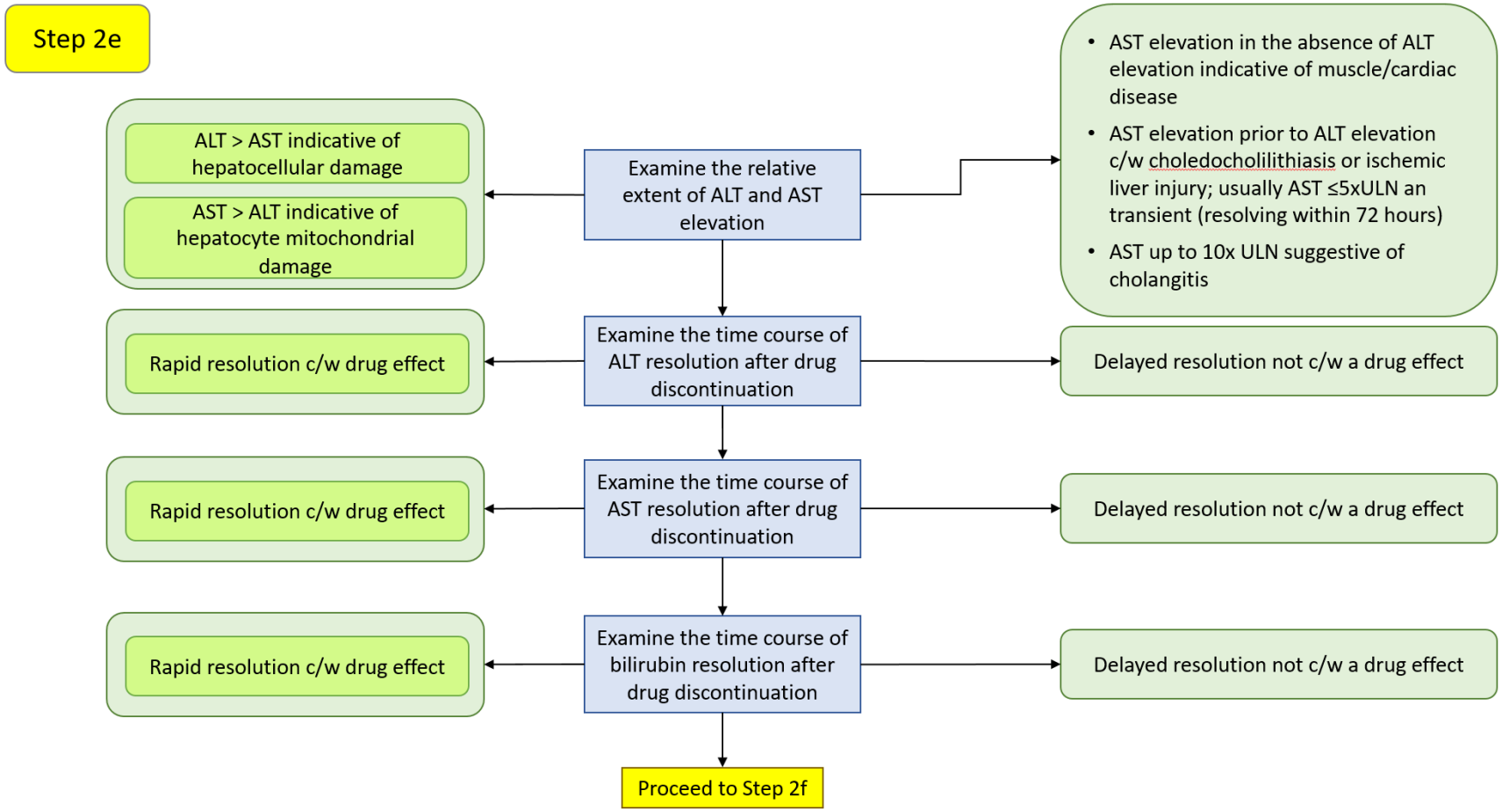
Interactive Safety Graphic – Hepatic Safety Explorer

Step 2d

Peak AST <12 weeks?	**Yes**: Serum AST activity is considered a less specific biomarker of liver function compared to ALT as it also appears in heart, skeletal muscle, kidneys, brain and red blood cells. AST occurs in two locations in the liver, the cytosol (20%) and mitochondria (80%) (Herlong & Mitchell 2012). In healthy individual, cytosolic AST represents the measurable isoenzyme in serum. AST and ALT levels are equally elevated in most hepatobiliary disorders, with the ALT level usually being somewhat higher than the AST level. When evaluating the time course of the AST elevation, the first 12 weeks from drug initiation is generally the period of greatest risk of drug-induced hepatotoxicity (Hunt et al. 2007). Elevations within the first couple weeks often reflect adaptation to drug load rather than an actual hepatotoxic effect, particularly when the daily dose is several hundred milligrams and higher (Dara et al. 2016). The majority of patients who experience AST elevations are not at risk of developing significant liver injury and will demonstrate resolution of the liver injury despite continued exposure to the drug as the liver develops immune tolerance or cellular adaptive responses (Dara et al. 2016). The ability to adapt appears to be a general phenomenon, while failure to adapt or defective adaptation leads to severe idiosyncratic injury. (Watkins 2005, Dara et al. 2016) Note that acute hepatobiliary obstruction or inflammation, such as caused by a gallstone, can result in an abrupt rise in transaminases, bilirubin and alkaline phosphatase (Green & Flamm 2002). A disproportionate increase of AST relative to ALT should prompt testing for creatine phosphokinase (CPK) that can assist in distinguishing between liver and muscle derived transaminases (EASL 2019).
	No: Although instances of delayed hepatotoxicity have been reported, peak AST levels in excess of 12 weeks are generally not consistent with a drug related effect.
Examine the time when AST reaches 3x, 5x, 10x and 20x ULN.	Evaluating the time points at which various multiples of the ULN are reached (rate of rise) provides information on the acute nature of the reaction. The greater the rate of rise, the more acute the onset of the toxic effect suggestive of a drug-related effect.

Examine the time course of the AST elevation relative to the bilirubin elevation	Only bilirubin elevations simultaneous with or soon after peak AST elevations may indicate loss of hepatic function due to drug-induced liver injury. A time interval exceeding four weeks between both peaks may also speak against a causal correlation (Merz et al. 2014). Proceed to Step 2e.
	Note: Apparent elevations in AST can occur when the blood sample hemolyzes. An aberrantly high potassium value is a reasonable flag for such hemolysis. (Trost 2015)

Interactive Safety Graphic – Hepatic Safety Explorer



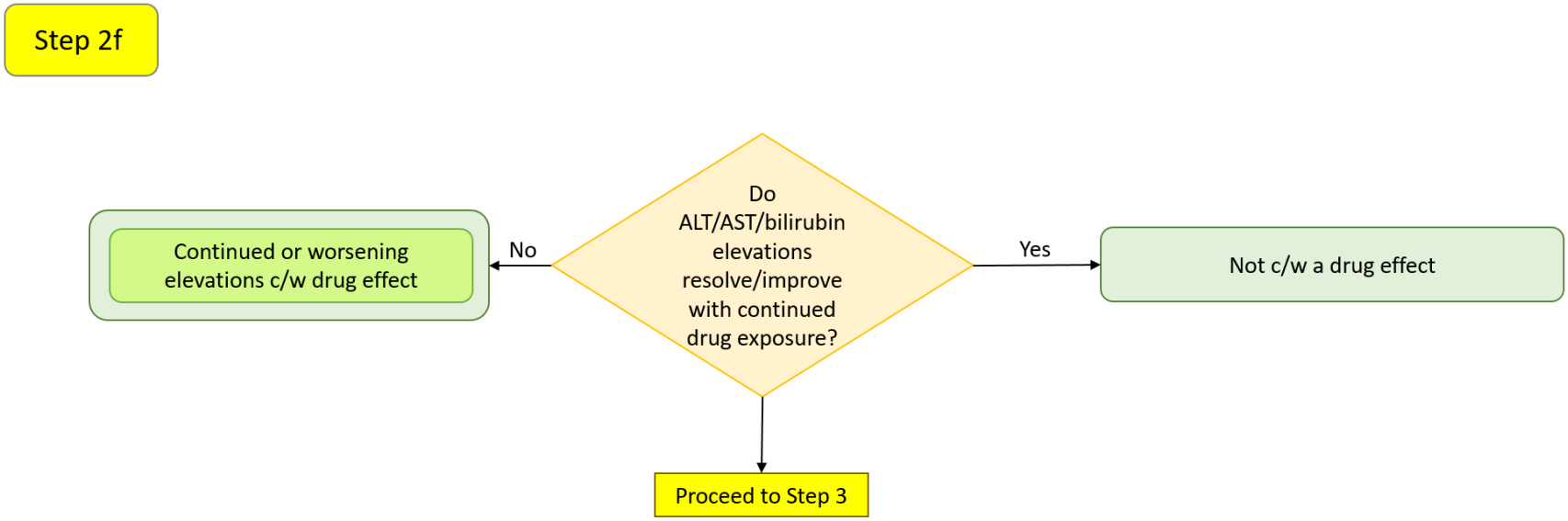
Interactive Safety Graphic – Hepatic Safety Explorer

Step 2e

Examine the relative extent of ALT and AST elevation	ALT elevation that exceeds the AST elevation is indicative of principally hepatocellular damage, whereas an AST elevation in excess of ALT elevation can suggest hepatocyte mitochondrial damage. Approximately 80% of AST activity is from mitochondria (Thapa & Walia 2007). Acute alcoholic hepatitis and cirrhosis can present with an AST/ALT ratio of 2:1 (Yang et al. 2014), since alcohol is a mitochondrial toxin, but other mitochondrial toxins can also result in a disproportionate elevation of AST to ALT. A ratio of AST/ALT greater than five, especially if ALT is normal or slightly elevated, is suggestive of injury to extrahepatic tissues, such as skeletal muscle in the case of rhabdomyolysis or strenuous exercise (Woreta & Alqahtani 2014). The AST : ALT ratio may be helpful in the recognition of alcoholic liver disease. If the AST is less than 400 IU/L, an AST : ALT ratio of more than 2 suggests alcoholic liver disease; a ratio greater than 3 is highly suggestive of alcoholic liver disease (Herlong & Mitchell 2012).
Examine the time course of ALT and AST resolution after drug discontinuation	AST and ALT are catabolized in the liver, primarily by cells in the reticuloendothethlial system. The plasma half-life of AST and ALT are 17± 5 hours and 47±10 hours, respectively. Thus, AST declines more rapidly than ALT, and ALT may be higher than AST in the recovery phase of injury (Woreta & Alqahtani 2014). Rapid resolution after drug discontinuation is consistent with a drug effect whereas a delayed resolution is not consistent with a drug effect. If the ALT declines at a rate of halving the ALT concentration every ~2 days, that is consistent with cessation of hepatocellular damage. A slower rate of decline suggests some degree of ongoing hepatocellular damage as the previously released ALT is eliminated while newly released ALT contributes to keeping the values elevated. The same is true for AST; however, in the absence of ongoing hepatocellular damage, AST values should decline by half every ~1 ½ days. **Note:** An ongoing elevation of the AST component exceeding ALT levels might reflect ongoing hepatocyte damage, release of mitochondrial AST, or possibly some zonal difference in AST vs ALT (Robles–Diaz et al. 2014).

Examine the time course of bilirubin resolution after drug discontinuation	Among subjects in a prospective study of DILI who had a total bilirubin ≥2.5 mg/dL, the median time from peak bilirubin to a 50% reduction was 14, 15 and 22 days in those with hepatocellular, cholestatic and mixed DILI, respectively and from peak bilirubin to <2.5 mg/dL was 30, 45 and 32 days in those with hepatocellular, cholestatic and mixed DILI, respectively (Chalasani et al. 2008). Time to resolution may be longer in elderly patients. Proceed to Step 2f.

Interactive Safety Graphic – Hepatic Safety Explorer



Interactive Safety Graphic – Hepatic Safety Explorer

Step 2f

Do ALT/AST/bilirubin elevations resolve/improve with continued drug exposure?	A drug may initially result in elevations of hepatic analytes, but the elevations may resolve with continued therapy due to adaptation (Shapiro & Lewis 2007, Abboud & Kaplowitz 2007, Dara et al. 2016). Alternatively, the abnormalities may have been unrelated to the drug.

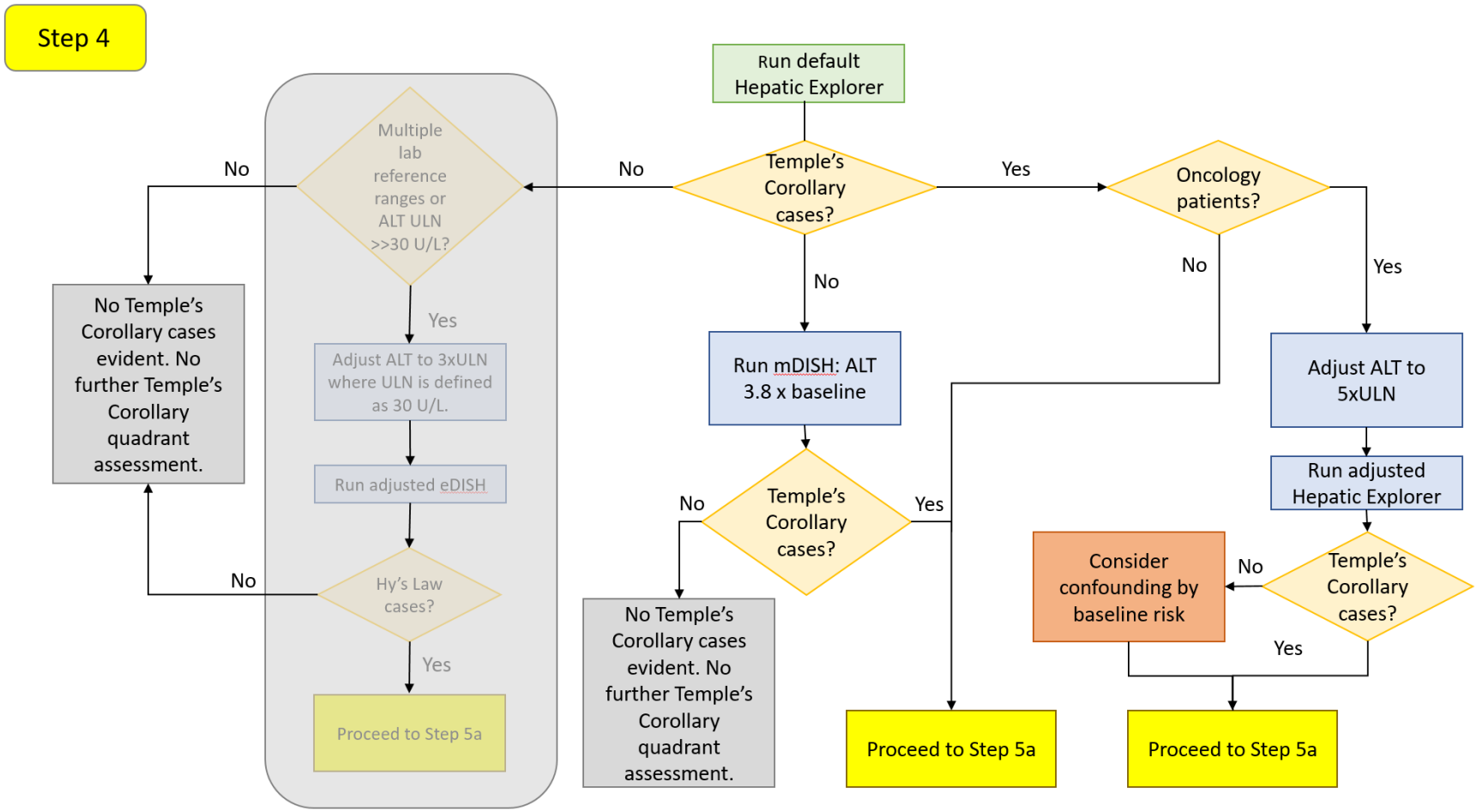
Additional Considerations

Extent of hepatic metabolism	Drugs with hepatic metabolism representing ≥50% of the elimination of the compound are more likely to be associated with ALT elevations ≥3 x ULN and liver failure (Lammert et al. 2010). Drugs metabolized via both Phase 1 (cytochrome P450 oxidation) and Phase 2 (conjugation) reactions have a higher propensity to cause ALT elevations ≥3 x ULN (Lammert et al. 2010).
Extent of ALT elevation relative to placebo	Moylen et al. (2012) reported that ALT elevations ≥3 x ULN that occurred in the study drug arm at a rate ≥1.2% greater than the placebo arm was associated with the subsequent development of a post-marketing EB_{05} score of ≥2 for liver-associated events with a positive predictive value of 71.4%.

Step 3

To be implemented in a future version.

Interactive Safety Graphic – Hepatic Safety Explorer



Note: The grayed-out portion is not available in Version 1.0 but is intended to be included in a later version.

Temple's Corollary Quadrant Evaluation

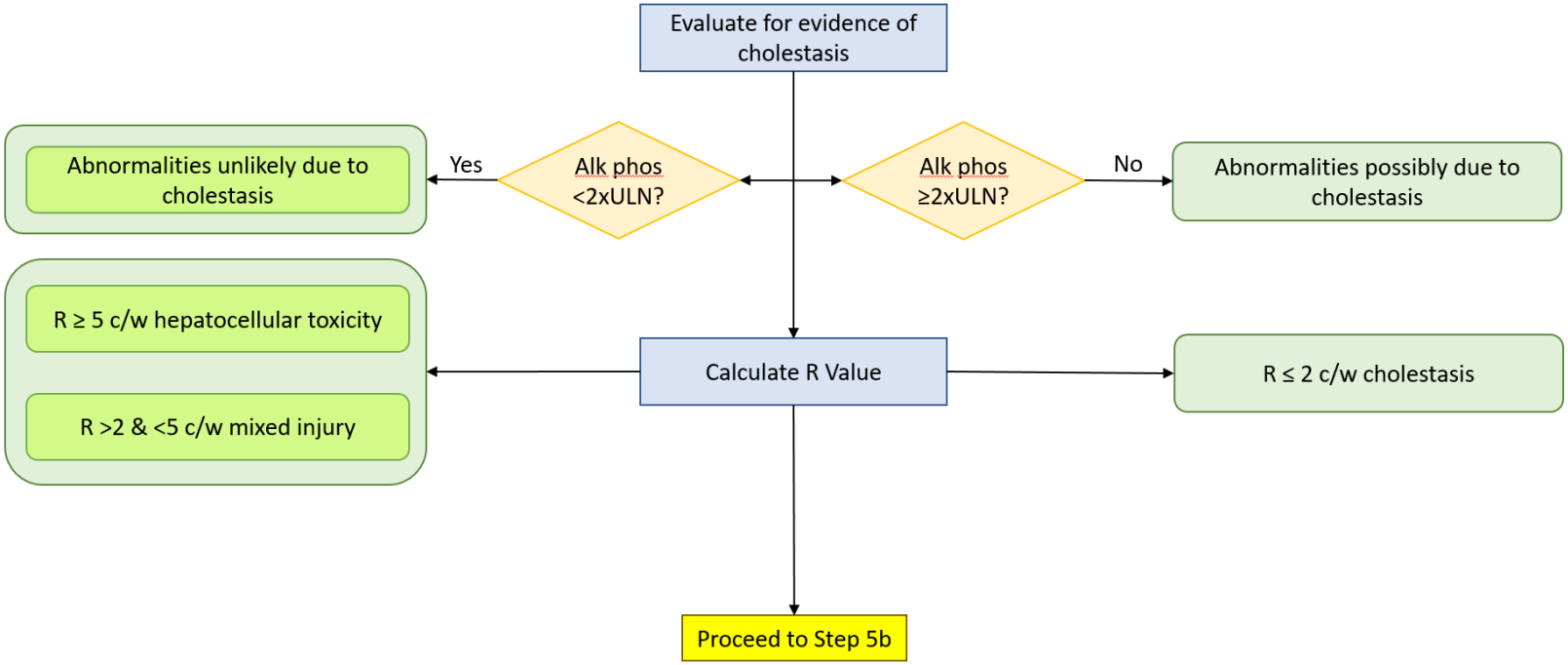
Step 4

Temple's Corollary cases?	**No**: After loading the dataset of interest, allow the tool to plot the results using the default settings. If no cases appear in the lower right Temple's Corollary quadrant, run an mDISH analysis. Patients may have clinically important changes in transaminase levels that don't meet Temple's Corollary definition when evaluated using the default fold-change from the upper limit of normal, particularly if patients begin drug treatment with relatively low values. The alternative is to perform the analysis on the basis of fold-change from baseline. The baseline-corrected approach, called mDISH (modified DISH), would be more sensitive to drug effects and is more consistent across laboratories (Ozer et al. 2010, Lin et al. 2012). Baseline-corrected data may also be used in populations with previous liver injury and abnormal liver biochemistry prior study drug administration (Aithal et al. 2011). For a generally healthy study population, the boundary thresholds in mDISH are recommended to be 3.8 x baseline for ALT and 4.8 x baseline for total bilirubin (Lin et al. 2012). Adjust the ALT Reference Line field accordingly. Using a single value to establish a baseline is not optimal considering the within-subject variation in liver tests (Merz et al. 2014). A more suitable determination of baseline may consist of two measurements at least two weeks but not more than two months apart. This tool presently only allows you to specify a single "baseline" value in the settings. However, if the dataset contains more than one pre-dose value, e.g., screening and baseline, adjust the "baseline" value in Settings and re-run the mDISH analysis. If the mDISH analysis yields no Temple's Corollary cases, then further analyses are not necessary. (Note this will change when the fold change from a fixed ULN function is implemented in a later version) If the mDISH analysis yields one or more Temple's Corollary cases, proceed to Step 5a.

	Yes: The appearance of cases in the Temple's Corollary quadrant could be the result of underlying risk factors in the population under study. Proceed to the next decision step: Oncology Patients?
Oncology patients?	**No**: Proceed to Step 5a.
	Yes: Oncology patients, particularly those with advanced disease, represent a population who often demonstrate elevated transaminase values at baseline due to extensive pretreatment and/or presence of liver metastases. Such patients may appear in the Temple's Corollary quadrant of a standard plot without necessarily experiencing drug-induced liver injury. A review of oncology patients, with and without evidence of liver metastases, recommended adjusting the ALT threshold (Parks et al. 2013). In patients without liver metastases, set the ALT threshold to 4.8 x ULN. In patients with liver metastases, set the ALT threshold to 5.5 x ULN. In patients either with or without known liver metastases, set the ALT threshold to 5.0 x ULN. Set these values in the ALT Reference Line field. If the adjusted thresholds result in the same Temple's Corollary cases, proceed to Step 5a. If the adjusted threshold result in the loss of cases from the Temple's Corollary quadrant, consider that their initial appearance in that quadrant could have been the result of confounding by the underlying disease process. Proceed to Step 5a.
	Note: Other conditions may result in elevations of transaminases; e.g., right heart failure/hypotension, connective tissue disorders involving the liver, inflammatory bowel disease, non-alcoholic steatohepatitis, viral hepatitis and use of total parenteral nutrition (Ozer et al. 2010). However, recommendations for adjusted ALT and bilirubin thresholds are not available for these situations. In the case of the ischemic hepatitis that develops with right heart failure, the elevation in bilirubin is due to unconjugated bilirubin in 24-81% of cases (Dunn et al. 1973), illustrating the utility of bilirubin fractionation. The user should consult a hepatologist for consideration of adjusting ALT thresholds when the dataset includes patients with these conditions.

Interactive Safety Graphic – Hepatic Safety Explorer

Step 5a

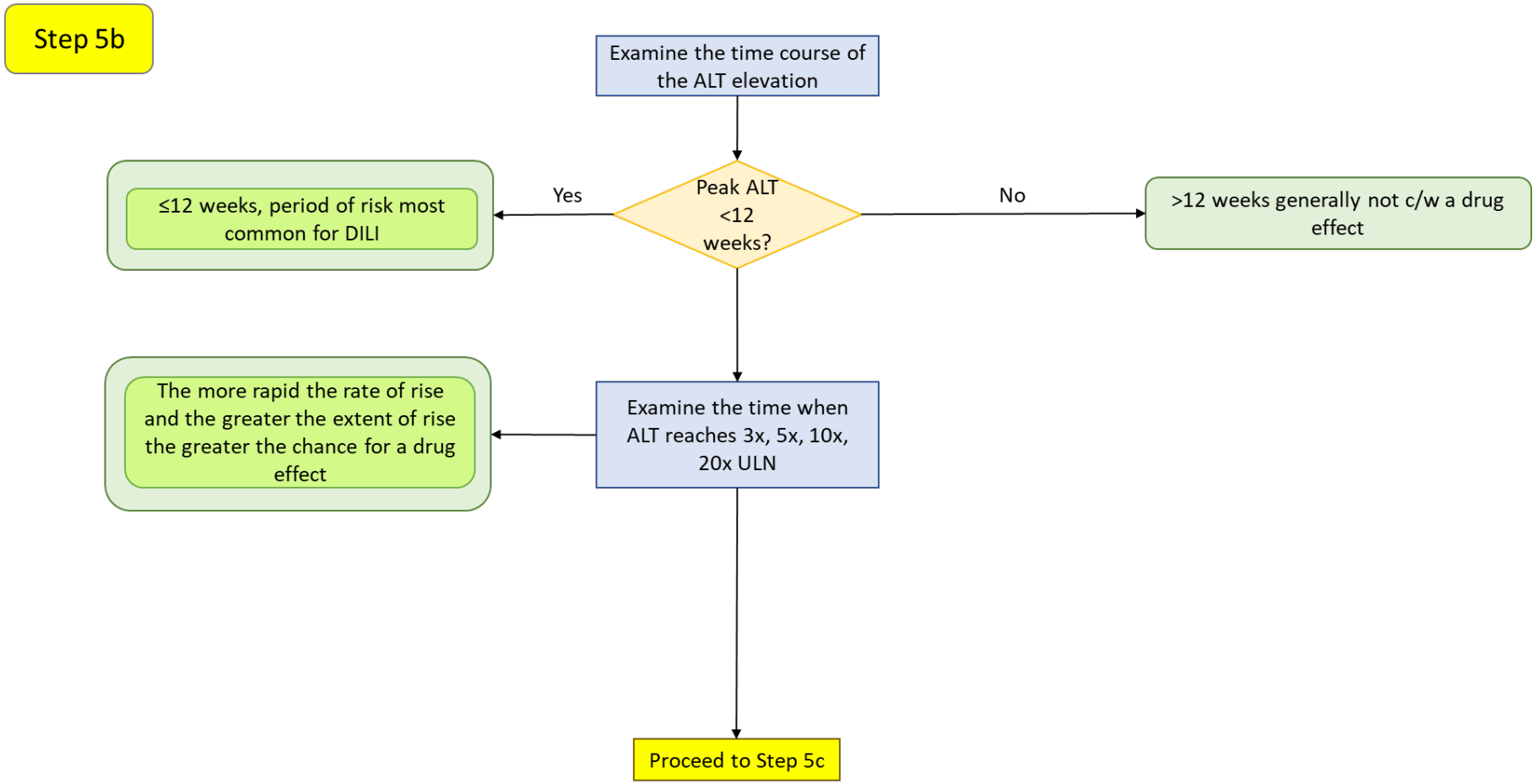


Interactive Safety Graphic – Hepatic Safety Explorer

Step 5a

Alk phos <2 x ULN?	**Yes**: Transaminase elevations in the absence of a concomitant elevation of alkaline phosphatase may be indicative of hepatocellular injury. The lack of an elevated alkaline phosphatase has been defined as <2x ULN (Avigan 2010). **No**: An elevated alkaline phosphatase level coincident with transaminase elevations may indicate a cholestatic source which could discount drug-related hepatocellular damage. However, this does not remove the possibility of drug-related cholestatic injury. **Note**: Alkaline phosphatase can be elevated by infiltrative diseases of the liver, tumors of hepatic and non-hepatic origin and bone diseases, including metastases to bone (AGA Clinical Practice Committee 2002).
Calculate R value	The R value (aka R ratio, R score) is calculated as [ALT/ULN]/[alkaline phosphatase/ULN] (Kullak-Ublick et al. 2017; Leise et al. 2014). R > 5 indicates hepatocellular injury R = 2-5 indicates mixed hepatocellular/cholestatic injury R < 2 indicates cholestatic injury A modified approach, called “new ratio” or nR, considers also the AST value in addition to the ALT value and uses whichever produced the highest fold change from the ULN (Robles-Diaz et al. 2014). Currently, this tool only calculates the R value based on ALT; calculation of the nR will need to be done manually at this time. Proceed to Step 5b.

Interactive Safety Graphic – Hepatic Safety Explorer



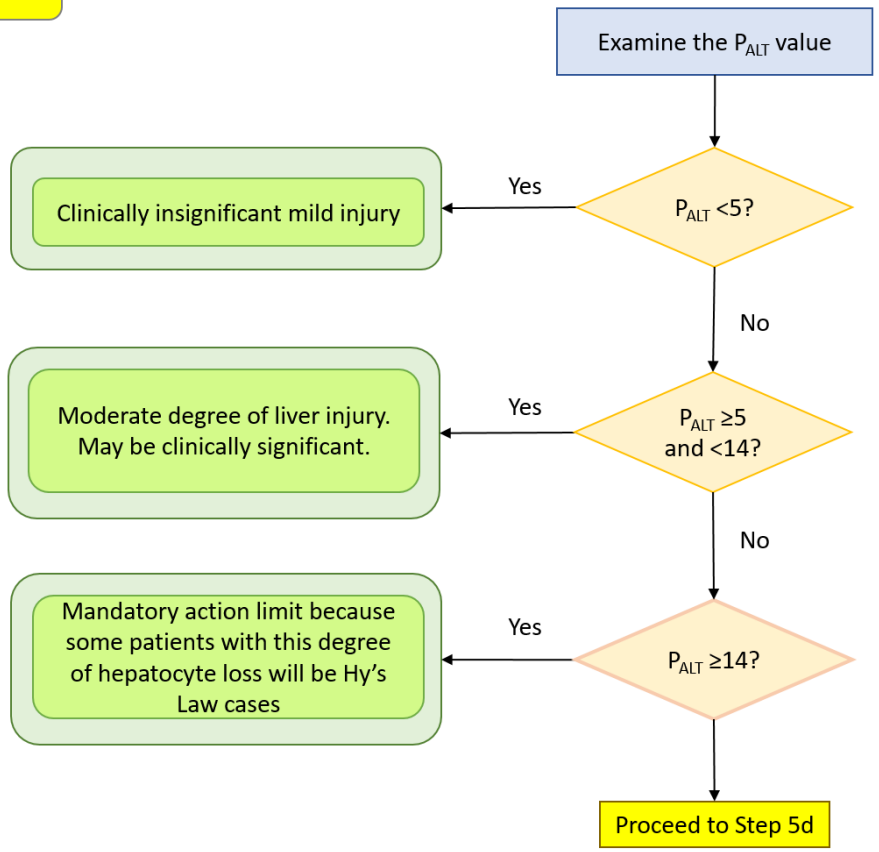
Interactive Safety Graphic – Hepatic Safety Explorer

Step 5b

Peak ALT <12 weeks?	**Yes**: When evaluating the time course of the ALT elevation, the first 12 weeks from drug initiation is generally the period of greatest risk of drug-induced hepatotoxicity (Hunt et al. 2007). Elevations within the first couple weeks often reflect adaptation to drug load rather than an actual hepatotoxic effect, particularly when the daily dose is several hundred milligrams and higher (Dara et al. 2016). The majority of patients who experience ALT elevations are not at risk of developing significant liver injury and will demonstrate resolution of the liver injury despite continued exposure to the drug as the liver develops immune tolerance or cellular adaptive responses (Dara et al. 2016). The ability to adapt appears to be a general phenomenon, while failure to adapt or defective adaptation leads to severe idiosyncratic injury. (Watkins 2005, Dara et al. 2016) Note that acute hepatobiliary obstruction or inflammation, such as caused by a gallstone, can result in an abrupt rise in transaminases and alkaline phosphatase (Green & Flamm 2002).
	No: Although instances of delayed hepatotoxicity have been reported, peak ALT levels in excess of 12 weeks is generally not consistent with a drug related effect.
Examine the time when ALT reaches 3x, 5x, 10x and 20x ULN.	Evaluating the time points at which various multiples of the ULN are reached (rate of rise) provides information on the acute nature of the reaction. The greater the rate of rise, the more acute the onset of the toxic effect suggestive of a drug-related effect. Proceed to Step 5c.

Interactive Safety Graphic – Hepatic Safety Explorer

Step 5c

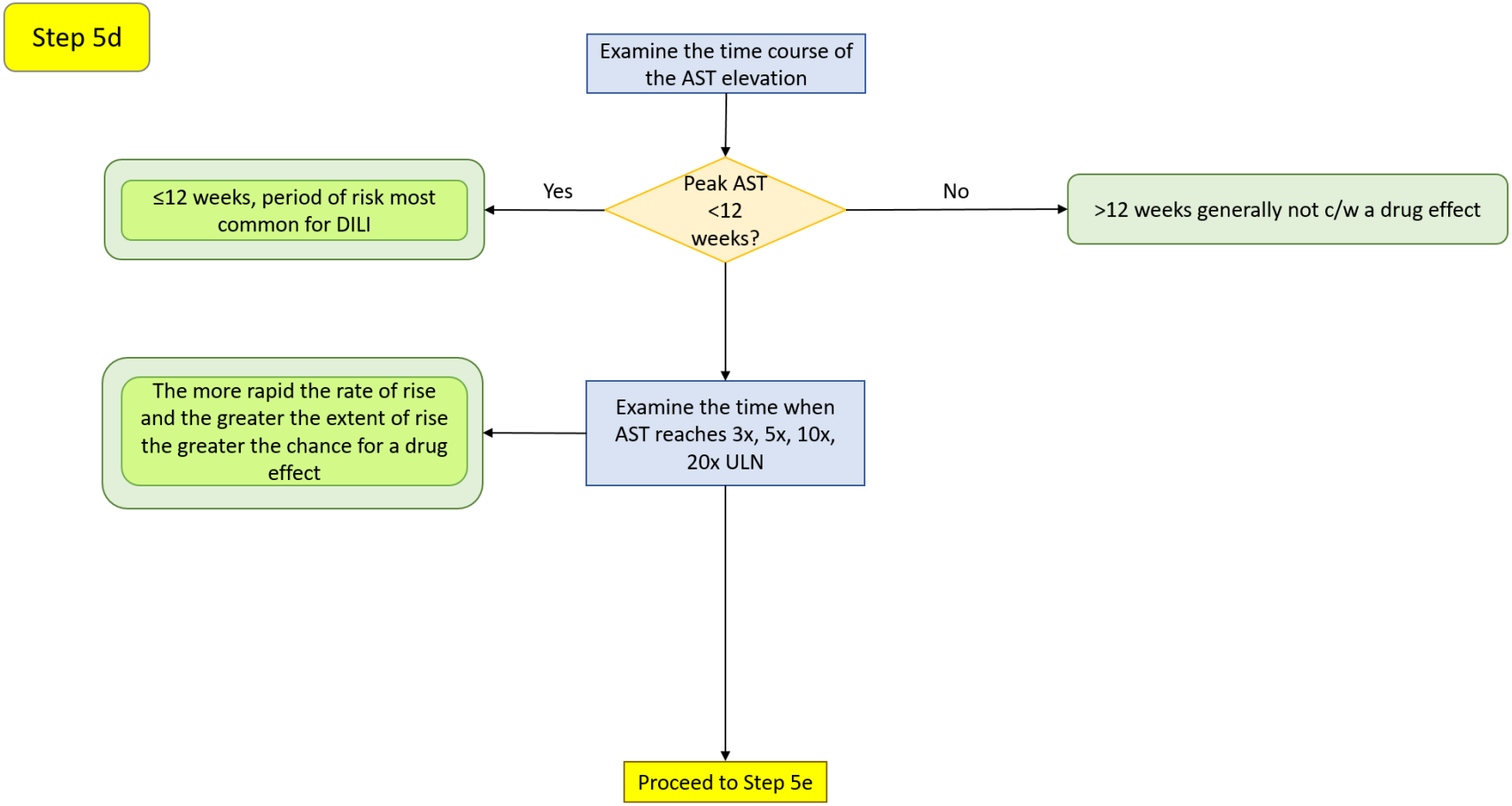


Interactive Safety Graphic – Hepatic Safety Explorer

Step 5c

P_{ALT} <5, ≥5 and <14 or ≥14?	P_{ALT}, calculated using an equation that uses peak ALT and the AUC of the serum ALT over time, provides an estimate of hepatocyte loss across a number of different time courses and patterns of injury (Chung et al. 2019). Based on acetaminophen overdose cases, it is reasonable to consider that 10% hepatocyte loss due to DILI as without clinical consequence and that >60% hepatocyte loss can be fatal. While multiple ALT values are preferable, it is possible to use P_{ALT} to estimate the maximum hepatocyte loss at any point during the course of elevated ALTs. **P_{ALT} values <5** represent clinically insignificant mild injury (95% upper CI limit of the amount of hepatocyte loss is <13%). **P_{ALT} values ≥5 and <14** represent a moderate degree of liver injury which may be clinically significant. Discontinue treatment if peak ALT >5x ULN for >2 weeks or ALT >3x ULN with fatigue, nausea, vomiting, right upper quadrant pain or tenderness, fever, rash, and/or eosinophilia (>5%) or INR >1.5. **P_{ALT} value of ≥14** (95% upper CI limit of the amount of hepatocyte loss is approaching 30%) may support liver injury sufficient to result in Hy's Law in some subjects (i.e., their serum bilirubin will exceed two times the ULN). **P_{ALT} values >30** are likely to lead to death (95% upper CI limit of the amount of hepatocyte loss is approaching 85%). When serum ALT elevations are observed in clinical trials, a common practice has been to search for correlations between drug exposure and the observed peak serum ALT values and thereby estimate safe exposure levels and hence optimal dosing schedules. Estimates of hepatocyte loss may be a better variable than peak serum ALT for this purpose. If an excellent correlation between drug exposure and hepatocyte loss is not evident, it is possible that interindividual differences in the underlying toxic mechanism(s) could be involved. This can now be addressed by incorporating quantitative systems toxicology models, such as DILIsym. Note that P_{ALT} was developed using models based on healthy livers. The estimate may not accurately predict hepatocyte loss in children and patients with preexisting liver diseases. Proceed to Step 5d.

Interactive Safety Graphic – Hepatic Safety Explorer



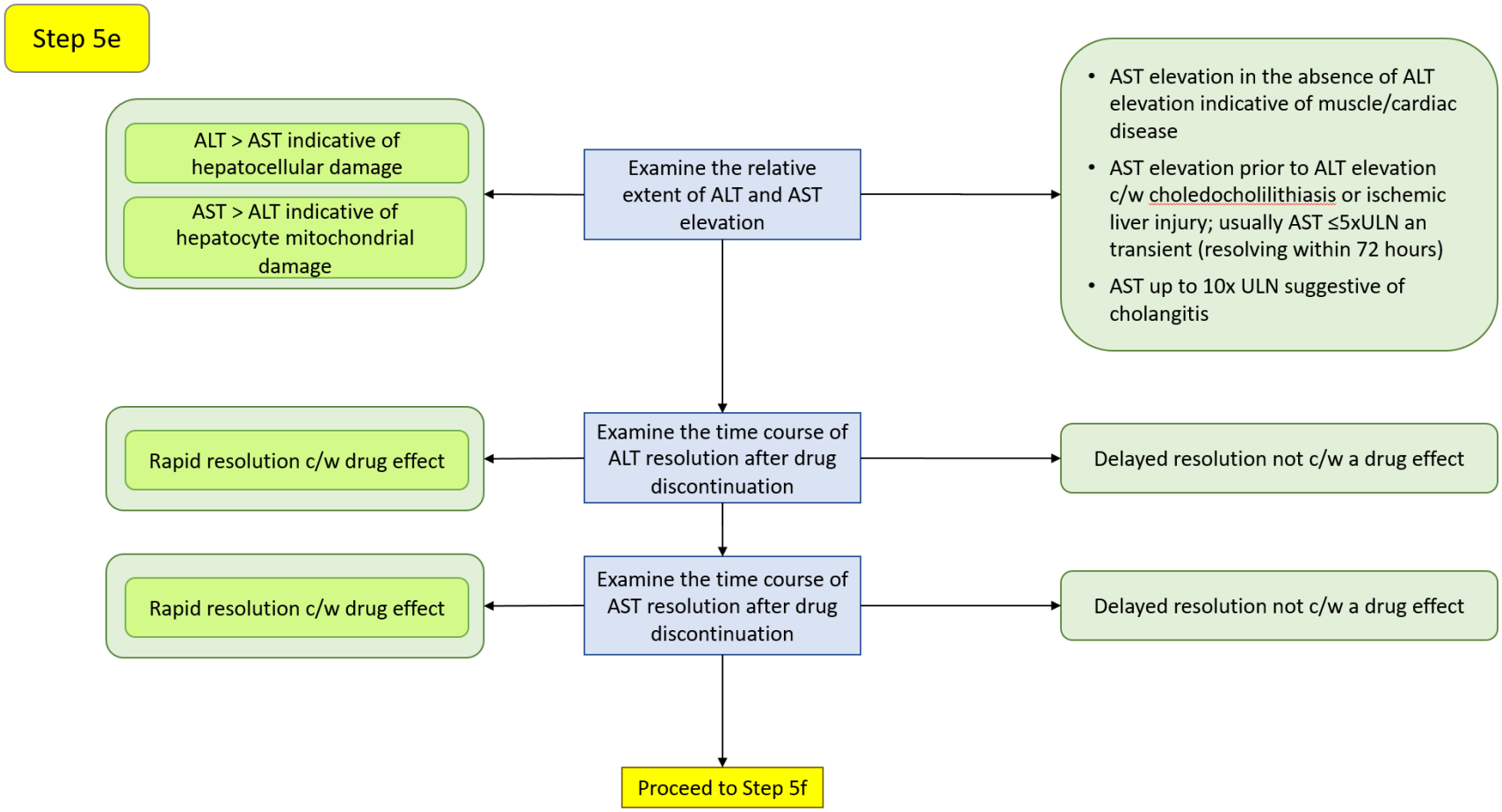
Interactive Safety Graphic – Hepatic Safety Explorer

Step 5d

Peak AST <12 weeks?	**Yes**: Serum AST activity is considered a less specific biomarker of liver function compared to ALT as it also appears in heart, skeletal muscle, kidneys, brain and red blood cells. AST occurs in two locations in the liver, the cytosol (20%) and mitochondria (80%) (Herlong & Mitchell 2012). In healthy individual, cytosolic AST represents the measurable isoenzyme in serum. AST and ALT levels are equally elevated in most hepatobiliary disorders, with the ALT level usually being somewhat higher than the AST level. When evaluating the time course of the AST elevation, the first 12 weeks from drug initiation is generally the period of greatest risk of drug-induced hepatotoxicity (Hunt et al. 2007). Elevations within the first couple weeks often reflect adaptation to drug load rather than an actual hepatotoxic effect, particularly when the daily dose is several hundred milligrams and higher (Dara et al. 2016). The majority of patients who experience AST elevations are not at risk of developing significant liver injury and will demonstrate resolution of the liver injury despite continued exposure to the drug as the liver develops immune tolerance or cellular adaptive responses (Dara et al. 2016). The ability to adapt appears to be a general phenomenon, while failure to adapt or defective adaptation leads to severe idiosyncratic injury. (Watkins 2005, Dara et al. 2016) Note that acute hepatobiliary obstruction or inflammation, such as caused by a gallstone, can result in an abrupt rise in transaminases and alkaline phosphatase (Green & Flamm 2002). A disproportionate increase of AST relative to ALT should prompt testing for creatine phosphokinase (CPK) that can assist in distinguishing between liver and muscle derived transaminases (EASL 2019).
	No: Although instances of delayed hepatotoxicity have been reported, peak AST levels in excess of 12 weeks is generally not consistent with a drug related effect.
Examine the time when AST reaches 3x, 5x, 10x and 20x ULN.	Evaluating the time points at which various multiples of the ULN are reached (rate of rise) provides information on the acute nature of the reaction. The greater the rate of rise, the more acute the onset of the toxic effect suggestive of a drug-related effect. Proceed to Step 5e.

	Note: Apparent elevations in AST can occur when the blood sample hemolyzes. An aberrantly high potassium value is a reasonable flag for such hemolysis. (Trost 2015)

Interactive Safety Graphic – Hepatic Safety Explorer

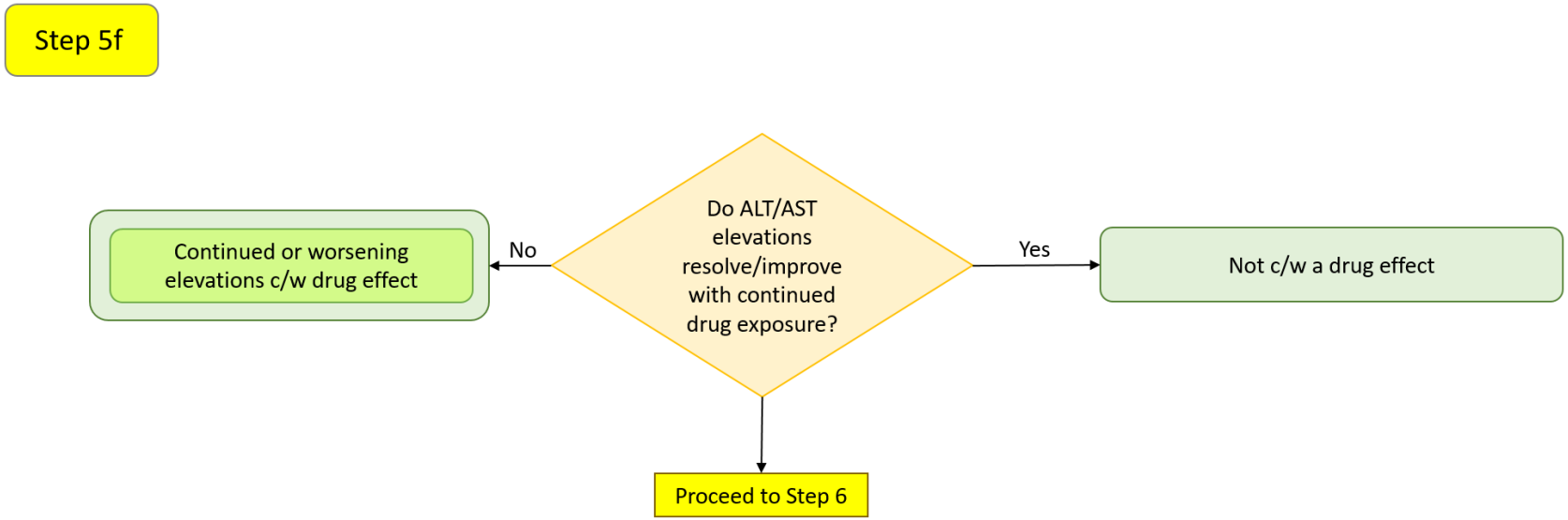


Interactive Safety Graphic – Hepatic Safety Explorer

Step 5e

Examine the relative extent of ALT and AST elevation	ALT elevation that exceeds the AST elevation is indicative of principally hepatocellular damage, whereas an AST elevation in excess of ALT elevation can suggest hepatocyte mitochondrial damage. However, an isolated elevation of AST values suggests a non-hepatic source of AST which often occurs artefactually due to release of AST from blood cells such as occurs in sample haemolysis (Botros & Sikaris 2013). Approximately 80% of AST activity is from mitochondria (Thapa & Walia 2007). Acute alcoholic hepatitis and cirrhosis can present with an AST/ALT ratio of 2:1 (Yang et al. 2014), since alcohol is a mitochondrial toxin, but other mitochondrial toxins can also result in a disproportionate elevation of AST to ALT. A ratio of AST/ALT greater than five, especially if ALT is normal or slightly elevated, is suggestive of injury to extrahepatic tissues, such as skeletal muscle in the case of rhabdomyolysis or strenuous exercise (Woreta & Alqahtani 2014). The AST : ALT ratio may be helpful in the recognition of alcoholic liver disease. If the AST is less than 400 IU/L, an AST : ALT ratio of more than 2 suggests alcoholic liver disease; a ratio greater than 3 is highly suggestive of alcoholic liver disease (Herlong & Mitchell 2012).
Examine the time course of ALT and AST resolution after drug discontinuation	AST and ALT are catabolized in the liver, primarily by cells in the reticuloendothethlial system. The plasma half-life of AST and ALT are 17± 5 hours and 47±10 hours, respectively. Thus, AST declines more rapidly than ALT, and ALT may be higher than AST in the recovery phase of injury (Woreta & Alqahtani 2014). Rapid resolution after drug discontinuation is consistent with a drug effect whereas a delayed resolution is not consistent with a drug effect. **Note:** An ongoing elevation of the AST component exceeding ALT levels might reflect ongoing hepatocyte damage, release of mitochondrial AST, or possibly some zonal difference in AST vs ALT (Robles–Diaz et al. 2014). Proceed to step 5f.

Interactive Safety Graphic – Hepatic Safety Explorer



Note: Step 6 to be implemented in a future version.

Interactive Safety Graphic – Hepatic Safety Explorer

Step 5f

Do ALT/AST elevations resolve/improve with continued drug exposure?	A drug may initially result in elevations of hepatic analytes, but the elevations may resolve with continued therapy due to adaptation (Shapiro & Lewis 2007, Abboud & Kaplowitz 2007, Dara et al. 2016). Alternatively, the abnormalities may have been unrelated to the drug.

Additional Considerations

Extent of hepatic metabolism	Drugs with hepatic metabolism representing ≥50% of the elimination of the compound are more likely to be associated with ALT elevations ≥3 x ULN and liver failure (Lammert et al. 2010). Drugs metabolized via both Phase 1 (cytochrome P450 oxidation) and Phase 2 (conjugation) reactions have a higher propensity to cause ALT elevations ≥3 x ULN (Lammert et al. 2010).
Extent of ALT elevation relative to placebo	Moylen et al. (2012) reported that ALT elevations ≥3 x ULN that occurred in the study drug arm at a rate ≥1.2% greater than the placebo arm was associated with the subsequent development of a post-marketing EB_{05} score of ≥2 for liver-associated events with a positive predictive value of 71.4%.

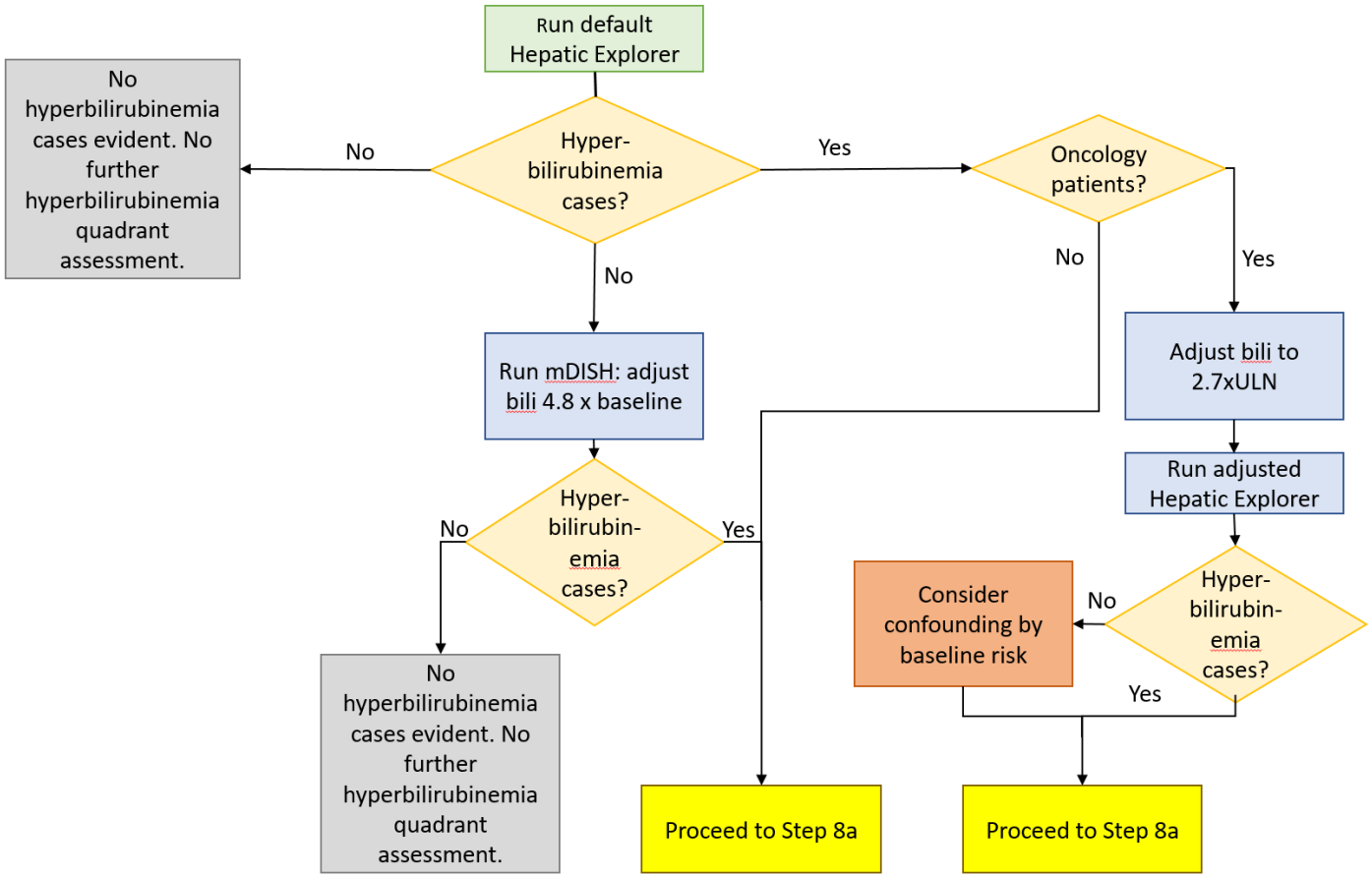
Interactive Safety Graphic – Hepatic Safety Explorer

Step 6

To be implemented in a future version.

Interactive Safety Graphic – Hepatic Safety Explorer

Step 7



Interactive Safety Graphic – Hepatic Safety Explorer

Hyperbilirubinemia Quadrant Evaluation

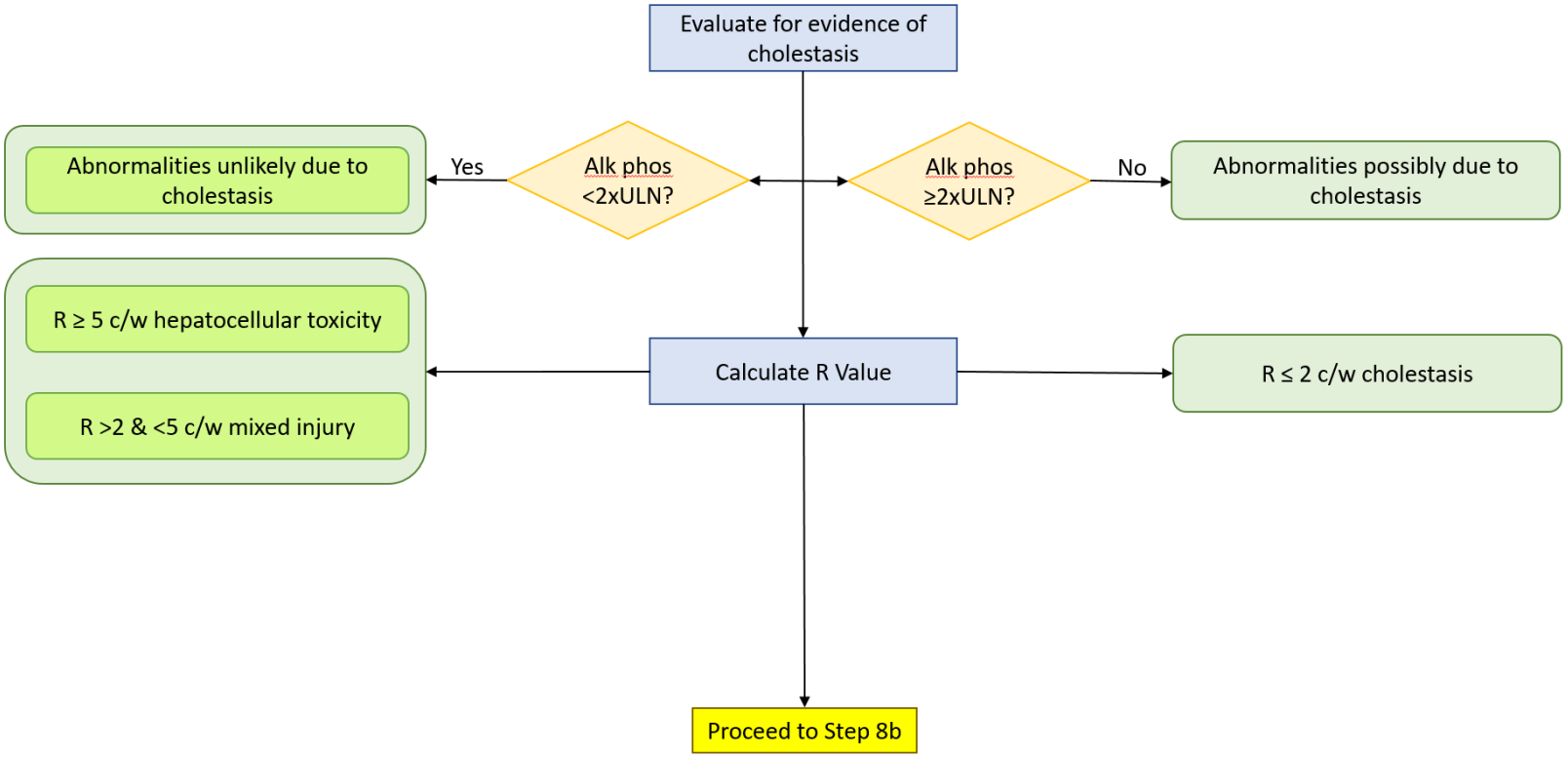
Step 7

Hyperbilirubinemia cases?	**No**: After loading the dataset of interest, allow the tool to plot the results using the default settings. If no cases appear in the upper left hyperbilirubinemia, run an mDISH analysis. Patients may have clinically important changes in bilirubin levels that don't meet hyperbilirubinemia definition when evaluated using the default fold-change from the upper limit of normal, particularly if patients begin drug treatment with relatively low values. The alternative is to perform the analysis on the basis of fold-change from baseline. The baseline-corrected approach, called mDISH (modified DISH), would be more sensitive to drug effects and is more consistent across laboratories (Ozer et al. 2010, Lin et al. 2012). Baseline-corrected data may also be used in populations with previous liver injury and abnormal liver biochemistry prior study drug administration (Aithal et al. 2011). For a generally healthy study population, the boundary thresholds in mDISH are recommended to be 4.8 x baseline for total bilirubin (Lin et al. 2012). Adjust the TB Reference Line fields accordingly. If the mDISH analysis yields no hyperbilirubinemia cases, then further analyses are not necessary. (Note this will change when the fold change from a fixed ULN function is implemented in a later version) If the mDISH analysis yields one or more hyperbilirubinemia cases, proceed to Step 8a.
	Yes: The appearance of cases in hyperbilirubinemia could be the result of underlying risk factors in the population under study. Proceed to the next decision step: Oncology Patients?
Oncology patients?	**No**: Proceed to Step 8a.
	Yes: Oncology patients, particularly those with advanced disease, represent a population who often demonstrate elevated bilirubin values at baseline due to extensive pretreatment and/or presence of liver metastases. Such patients may appear in the hyperbilirubinemia quadrant of a standard plot without necessarily experiencing drug-induced liver injury. A review of oncology patients, with and without evidence of liver metastases, recommended adjusting the total bilirubin thresholds (Parks et al. 2013). In patients without liver metastases, set the bilirubin

	threshold to 2.5 x ULN. In patients with liver metastases, set the bilirubin threshold to 3.0 x ULN. In patients either with or without known liver metastases, set the bilirubin threshold to 2.7 x ULN. Set these values in the TB Reference Line fields. If the adjusted thresholds result in the same hyperbilirubinemia cases, proceed to Step 8a. If the adjusted threshold result in the loss of cases from the hyperbilirubinemia quadrant, consider that their initial appearance could have been the result of confounding by the underlying disease process. Proceed to Step 8a.
	Note: Other conditions may result in elevations of total bilirubin; e.g., right heart failure/hypotension, connective tissue disorders involving the liver, inflammatory bowel disease, non-alcoholic steatohepatitis, viral hepatitis and use of total parenteral nutrition (Ozer et al. 2010). However, recommendations for adjusted ALT and bilirubin thresholds are not available for these situations. In the case of the ischemic hepatitis that develops with right heart failure, the elevation in bilirubin is due to unconjugated bilirubin in 24-81% of cases (Dunn et al. 1973), illustrating the utility of bilirubin fractionation. The user should consult a hepatologist for consideration of adjusting total bilirubin thresholds when the dataset includes patients with these conditions.

Interactive Safety Graphic – Hepatic Safety Explorer

Step 8a

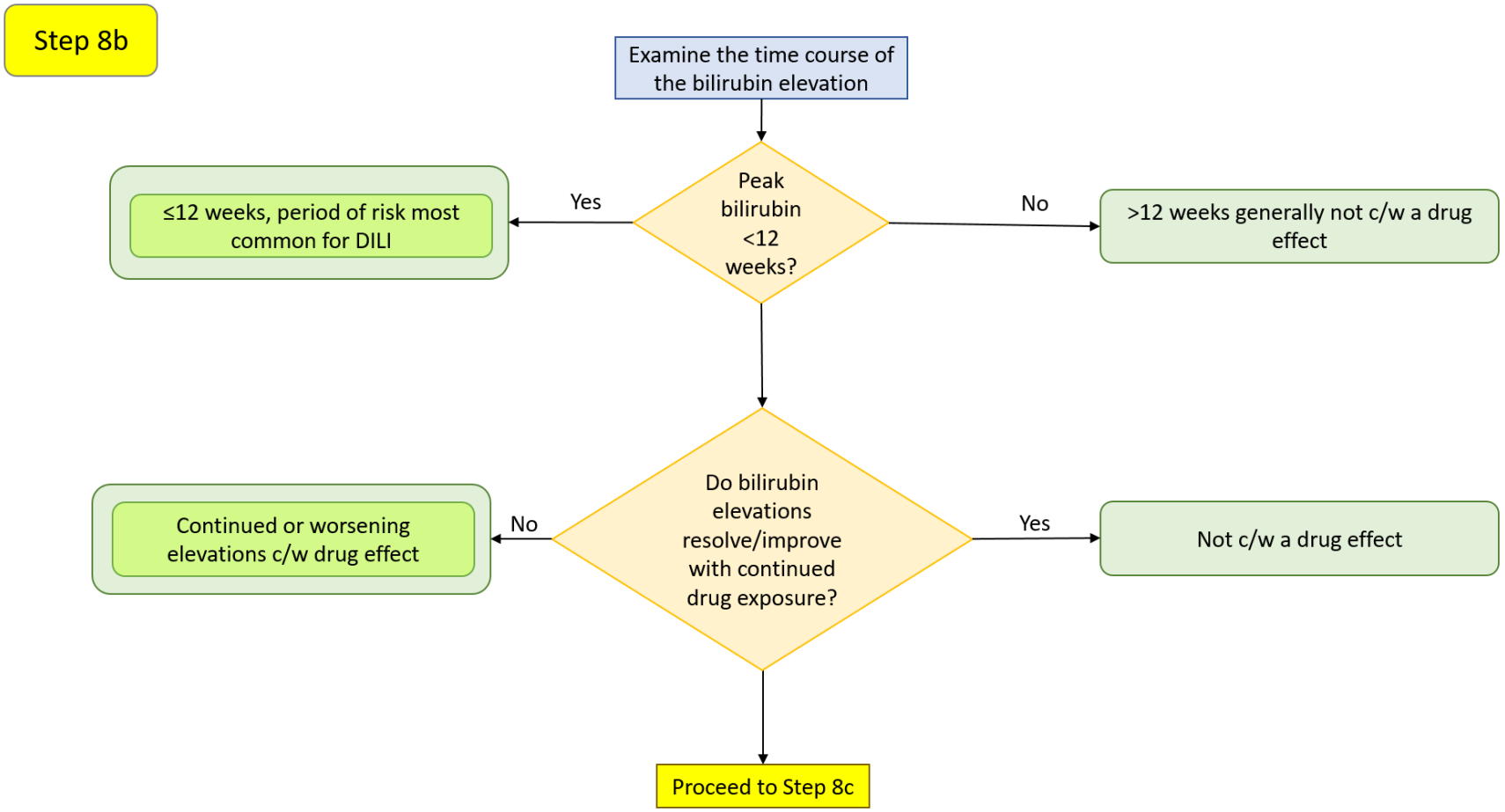


Interactive Safety Graphic – Hepatic Safety Explorer

Step 8a

Alk phos <2 x ULN?	**Yes**: Bilirubin elevations in the absence of a concomitant elevation of alkaline phosphatase may be indicative of hepatocellular injury. The lack of an elevated alkaline phosphatase has been defined as <2x ULN (Avigan 2010). **No**: An elevated alkaline phosphatase level coincident with bilirubin elevations may indicate a cholestatic source of the bilirubin elevation which could discount drug-related hepatocellular damage. However, this does not remove the possibility of drug-related cholestatic injury. **Note**: Alkaline phosphatase can be elevated by infiltrative diseases of the liver, tumors of hepatic and non-hepatic origin and bone diseases, including metastases to bone (AGA Clinical Practice Committee 2002). In such circumstances, these factors confound the ability to assess whether bilirubin elevations may be due to a cholestatic process.
Calculate R value	The R value (aka R ratio, R score) is calculated as [ALT/ULN]/[alkaline phosphatase/ULN] (Kullak-Ublick et al. 2017; Leise et al. 2014). R > 5 indicates hepatocellular injury R = 2-5 indicates mixed hepatocellular/cholestatic injury R < 2 indicates cholestatic injury A modified approach, called “new ratio” or nR, considers also the AST value in addition to the ALT value and uses whichever produced the highest fold change from the ULN (Robles-Diaz et al. 2014). Currently, this tool only calculates the R value based on ALT; calculation of the nR will need to be done manually at this time.

Interactive Safety Graphic – Hepatic Safety Explorer

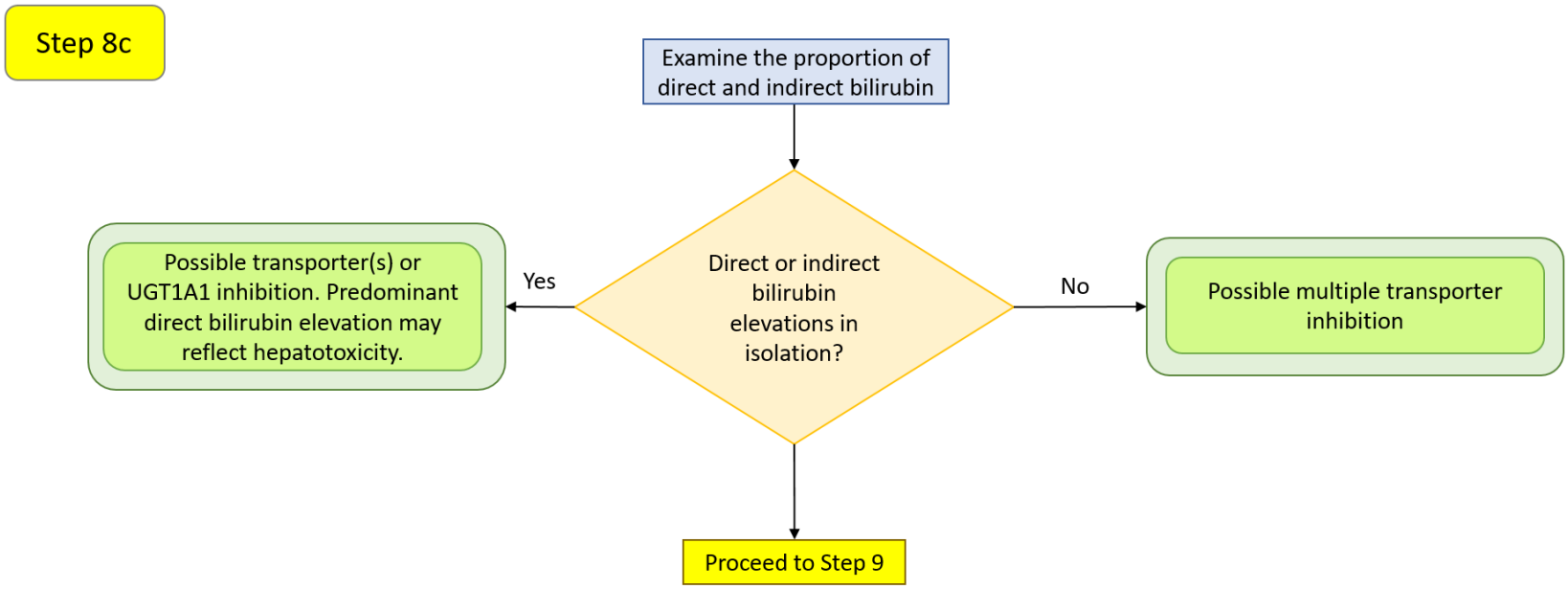


Interactive Safety Graphic – Hepatic Safety Explorer

Step 8b

Peak bilirubin <12 weeks?	**Yes**: The period of greatest risk of drug-induced hepatotoxicity (Hunt et al. 2007). Note that acute hepatobiliary obstruction or inflammation, such as caused by a gallstone, can result in an abrupt rise in bilirubin and alkaline phosphatase (Green & Flamm 2002).
	No: Although instances of delayed hepatotoxicity have been reported, peak bilirubin levels in excess of 12 weeks is generally not consistent with a drug related effect.
Do bilirubin elevations resolve/improve with continued drug exposure?	A drug may initially result in elevations of hepatic analytes, but the elevations may resolve with continued therapy due to adaptation (Shapiro & Lewis 2007, Abboud & Kaplowitz 2007, Dara et al. 2016). Alternatively, the abnormalities may have been unrelated to the drug.

Interactive Safety Graphic – Hepatic Safety Explorer



Note: Step 9 to be implemented in a future version.

Interactive Safety Graphic – Hepatic Safety Explorer

Step 8c

Is the bilirubin elevation due primarily to an increase in either unconjugated (indirect) bilirubin or conjugated bilirubin (direct), or are both components elevated?	Isolated elevations in unconjugated (indirect) bilirubin may be caused by drugs that inhibit the transporters OATP1B1 / OATP1B3, responsible for the uptake of unconjugated bilirubin into hepatocytes. In addition, or in lieu of, elevation of unconjugated bilirubin may be due to inhibition of UGT1A1, the enzyme responsible for conjugating bilirubin. (Chu et al. 2017) All evaluations of elevated levels of unconjugated bilirubin should consider increased production of unconjugated bilirubin by hemolysis. (Ah et al. 2008) Elevations of total bilirubin are generally comprised of conjugated (direct) bilirubin in instances of drug-induced liver injury (Hunt et al. 2007). Elevation of bilirubin generally not accompanied by evidence of cholestasis, e.g., elevated alkaline phosphatase, until later stage. (Trost 2015) Isolated elevations in conjugated (direct) bilirubin may be caused by drugs that inhibit the efflux transporter MRP2, responsible for transporting conjugated bilirubin across the canalicular membrane into bile. (Chu et al. 2017) Elevation of both unconjugated and conjugated bilirubin may result from inhibition of multiple transporters, e.g., OATP1B1, OATP1B3 and MRP2, as well as the possible contribution of inhibition of the UGT1A1 enzyme. (Chu et al. 2017)

Step 9

To be implemented in a future version

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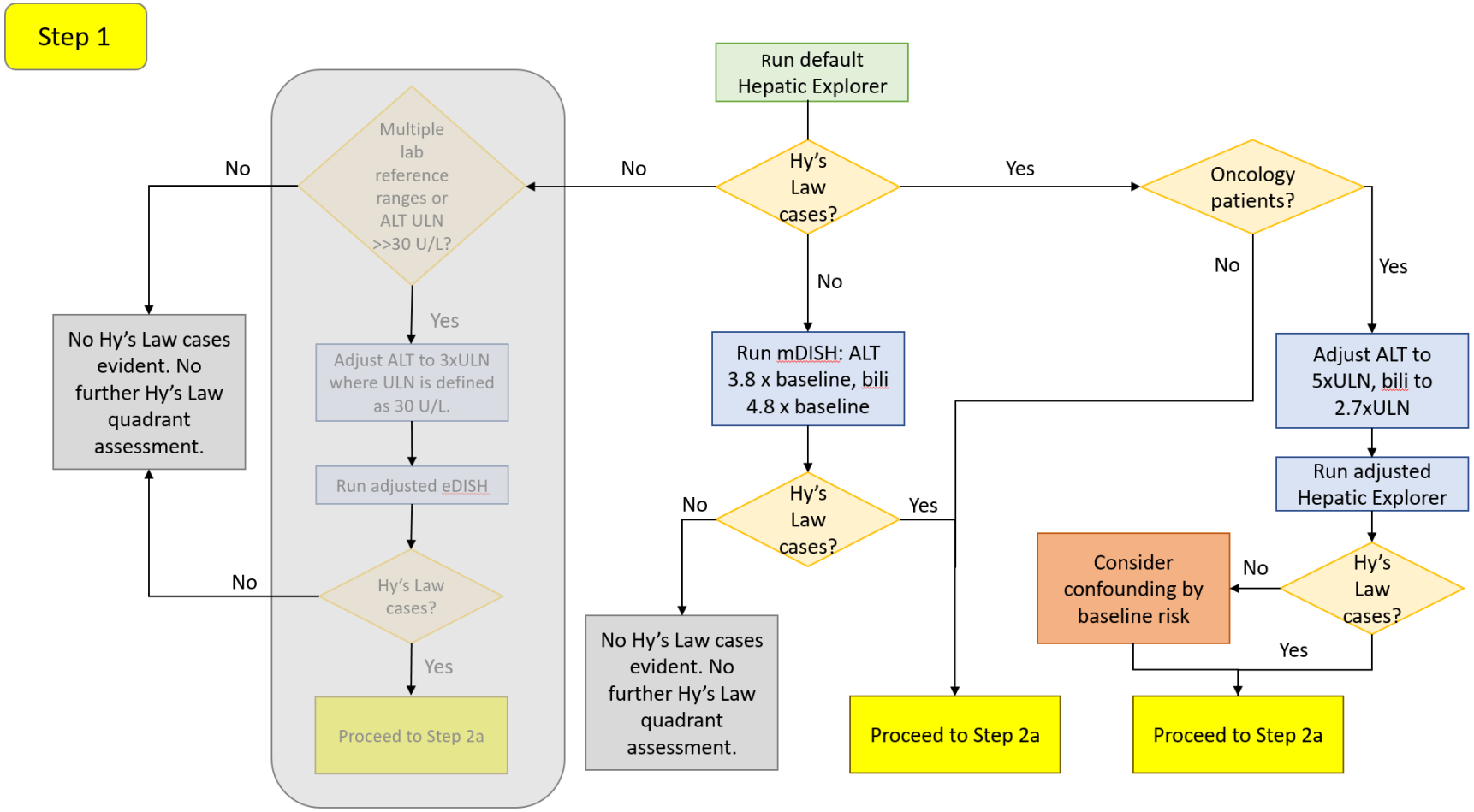
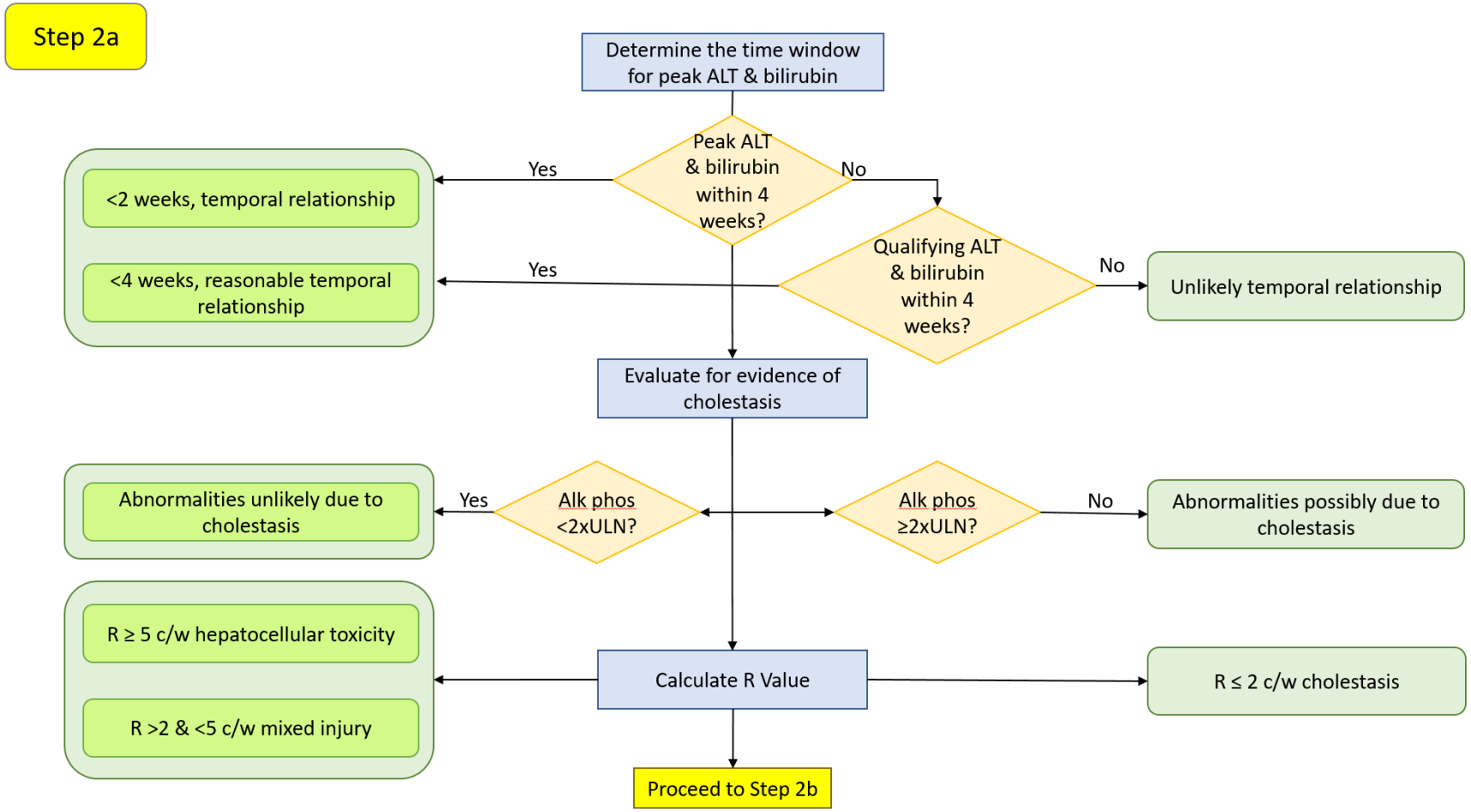
Interactive Safety Graphic – Hepatic Safety Explorer

Appendix 1: Consolidated Potential Hy's Law Case Workflow



Interactive Safety Graphic – Hepatic Safety Explorer



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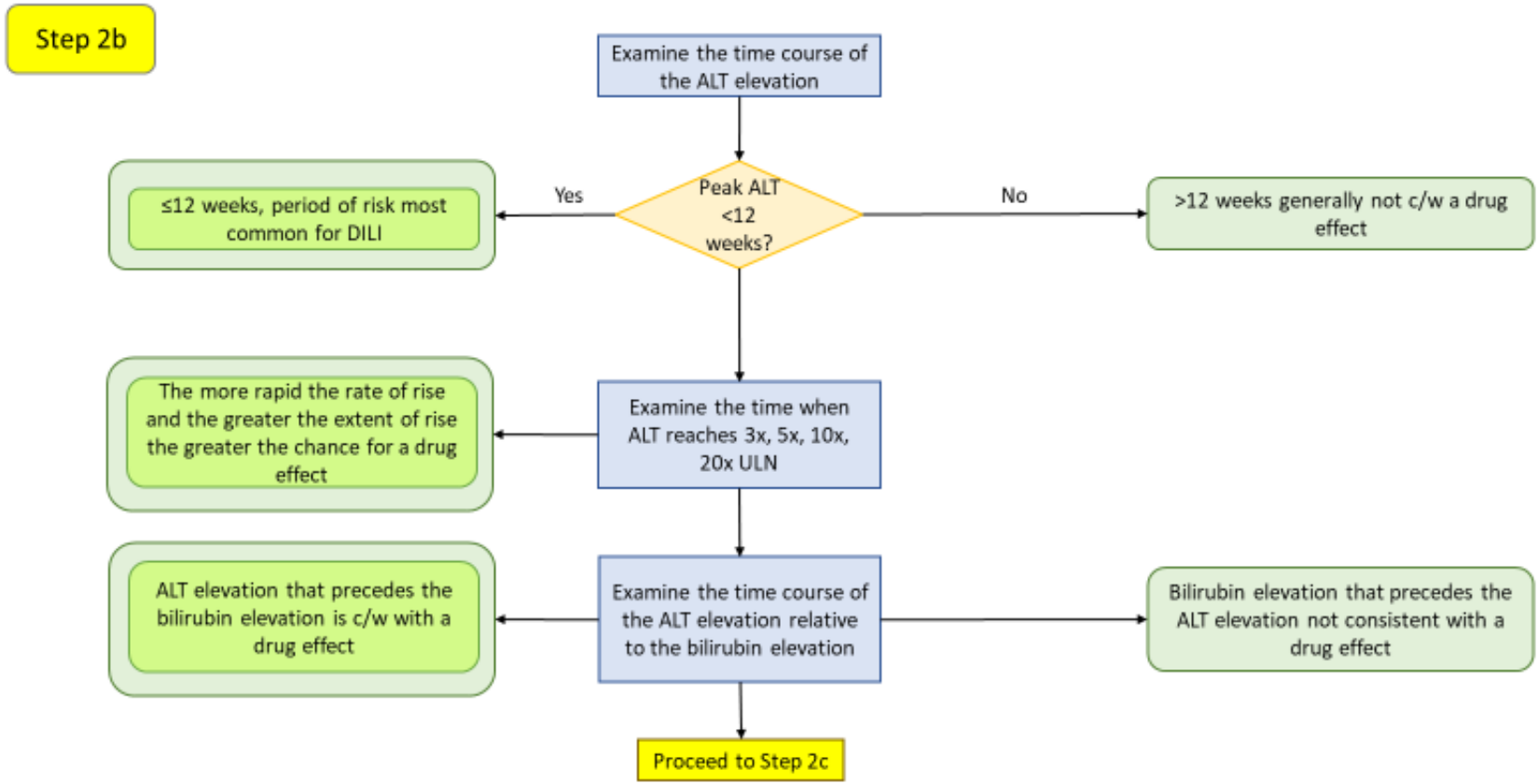

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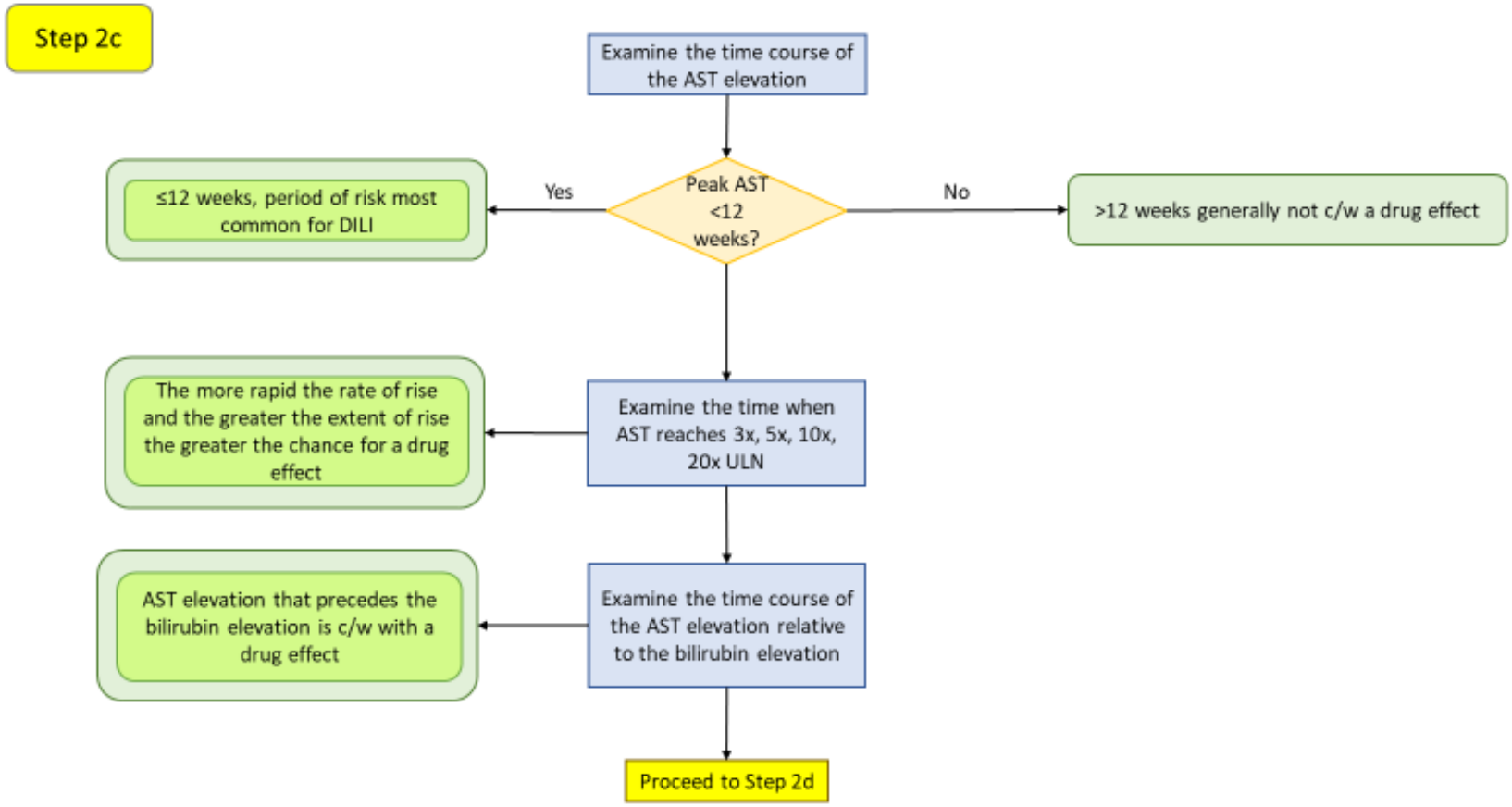

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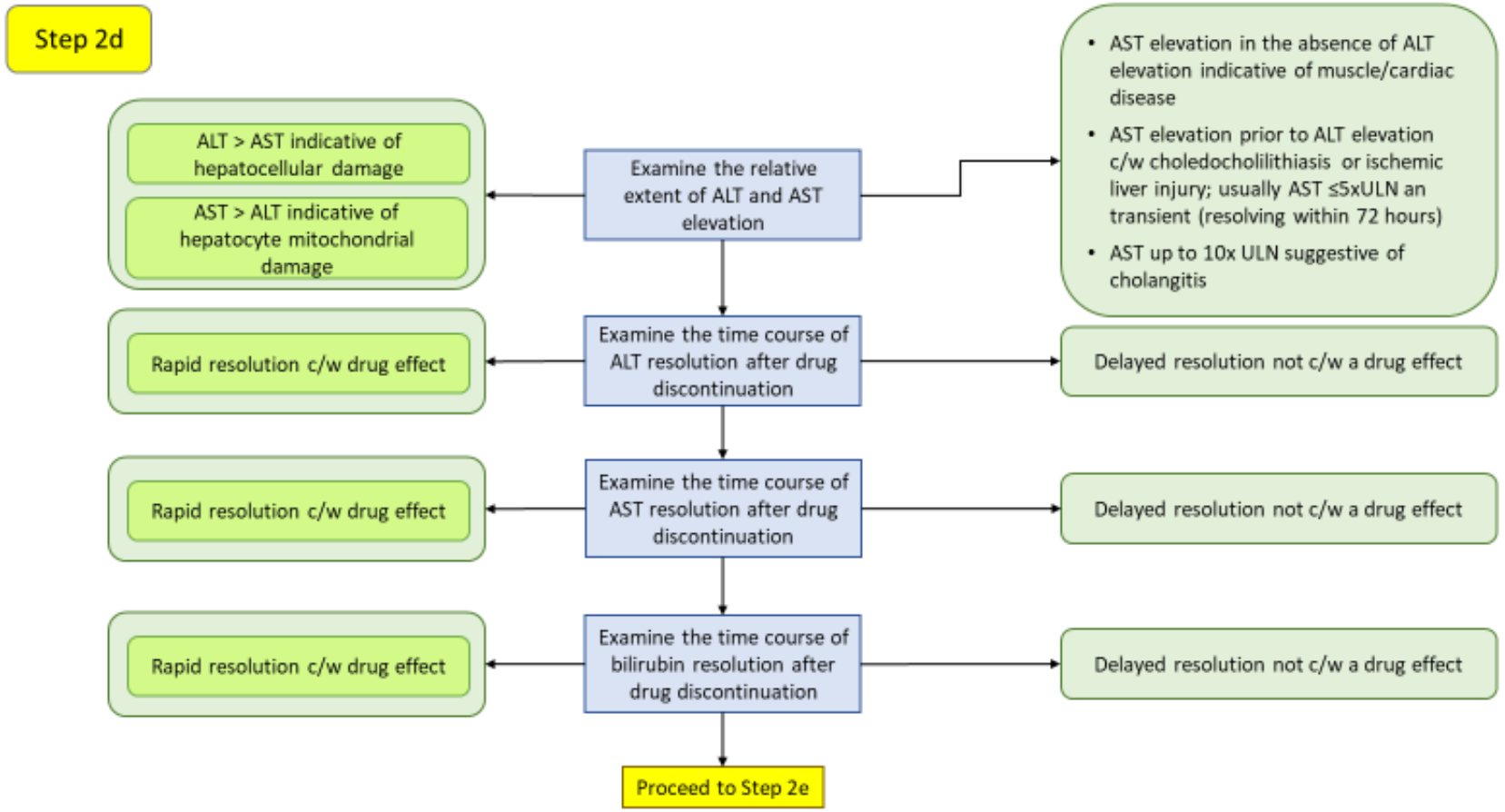

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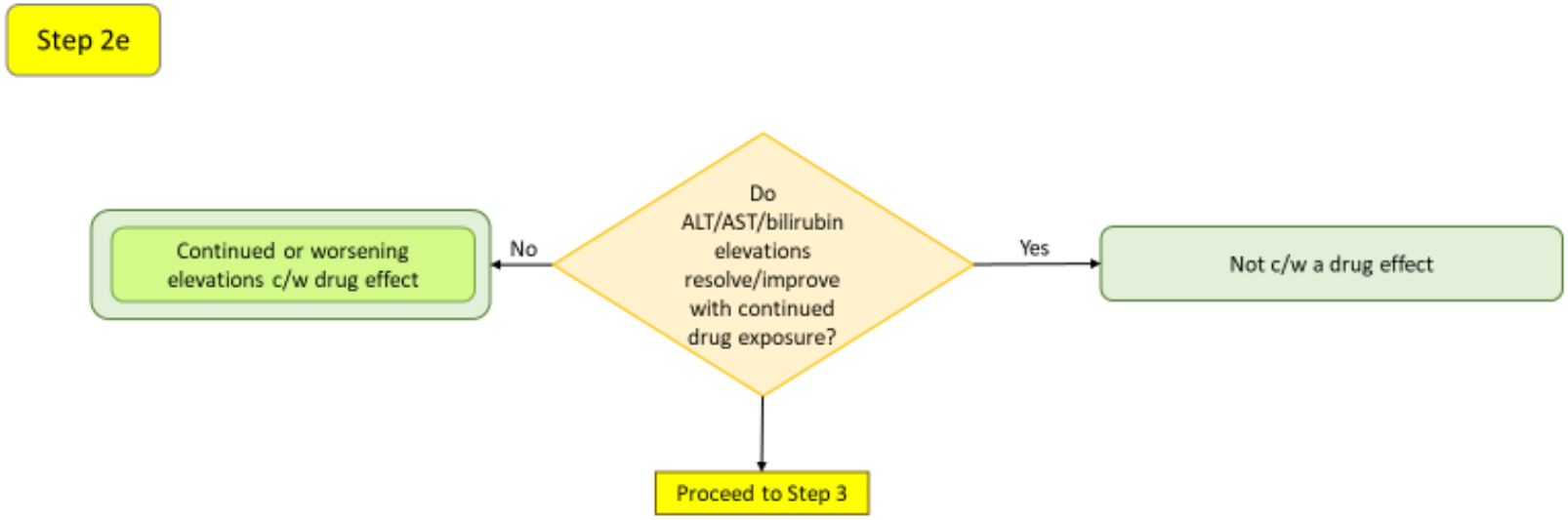
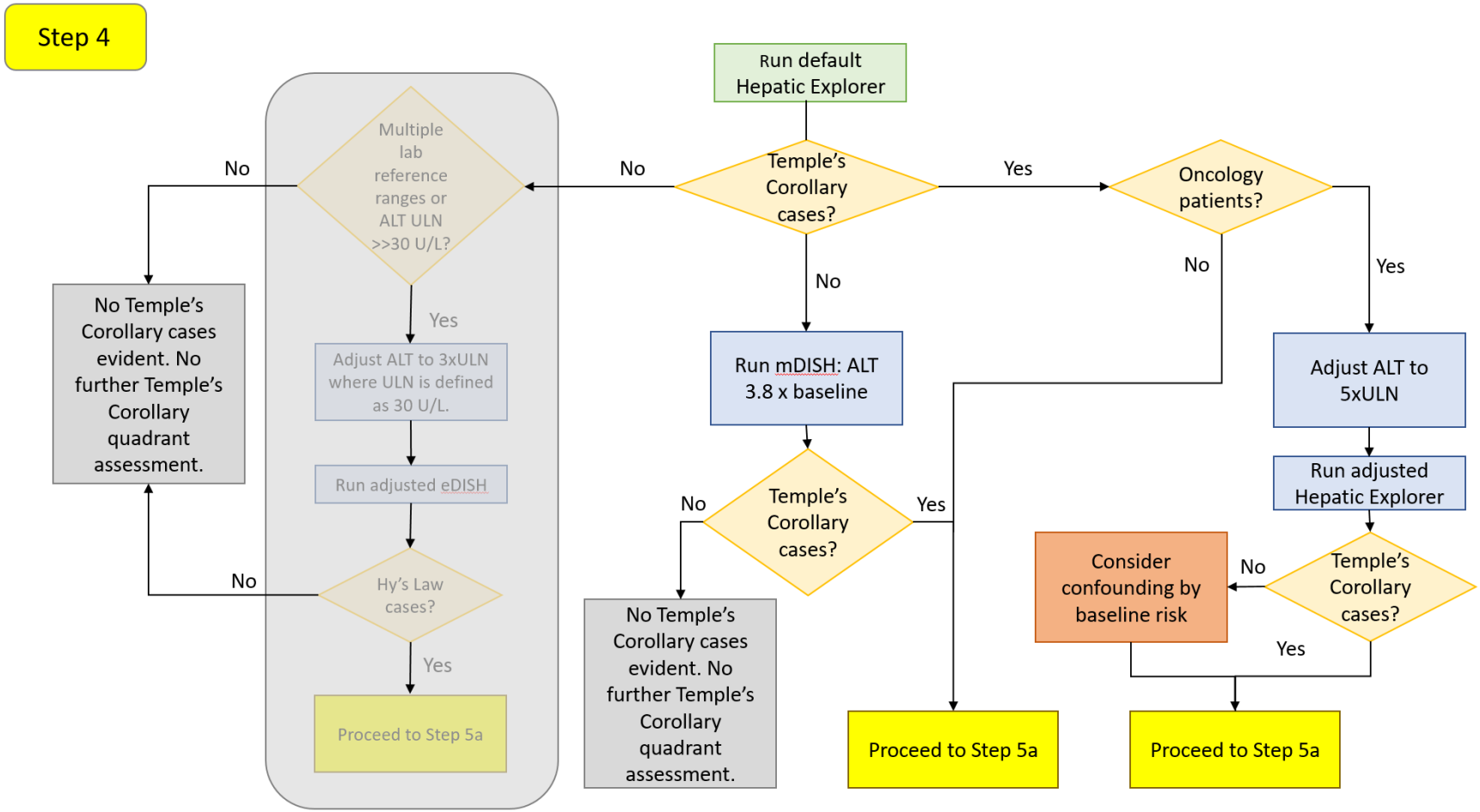


Note: Step 3 to be implemented in a future version

Interactive Safety Graphic – Hepatic Safety Explorer

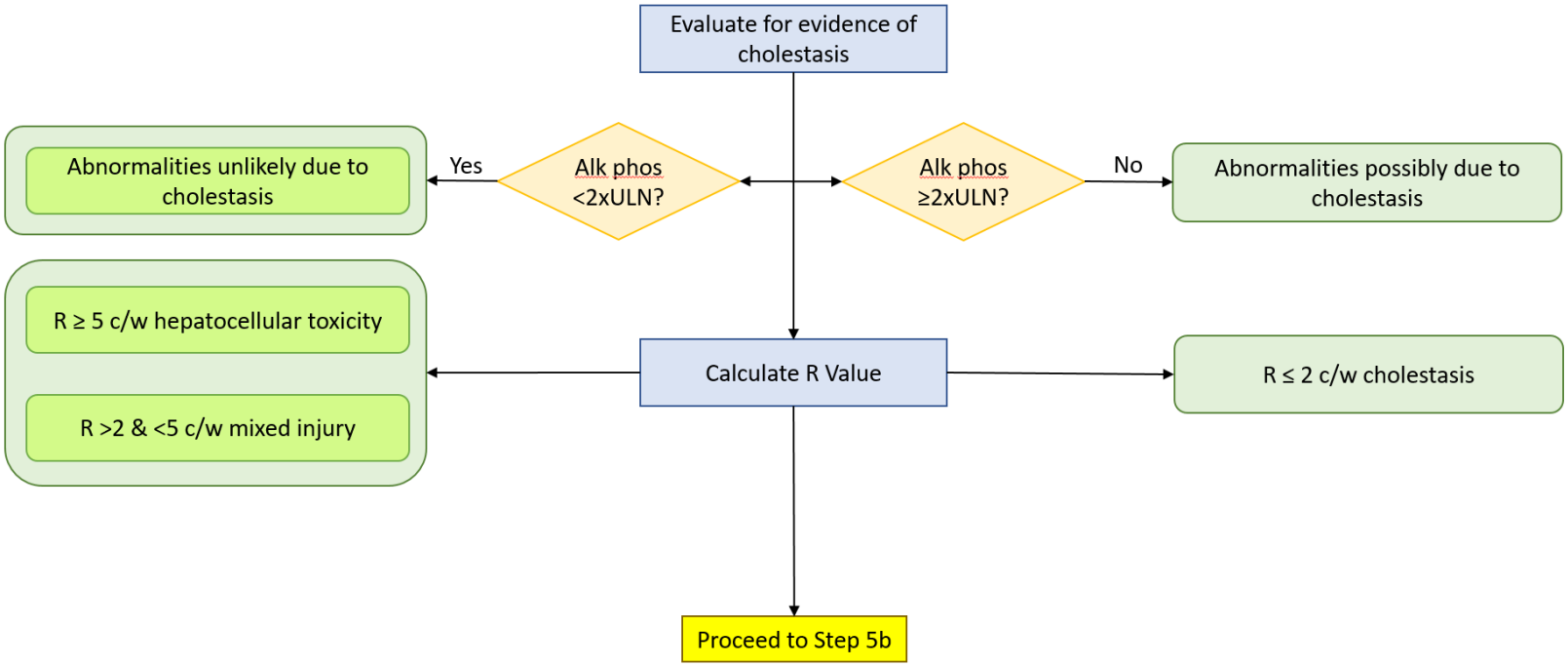
Appendix 2: Consolidated Temple's Corollary Case Workflow



Note: The grayed-out portion is not available in Version 1.0 but is intended to be included in a later version.

Interactive Safety Graphic – Hepatic Safety Explorer

Step 5a



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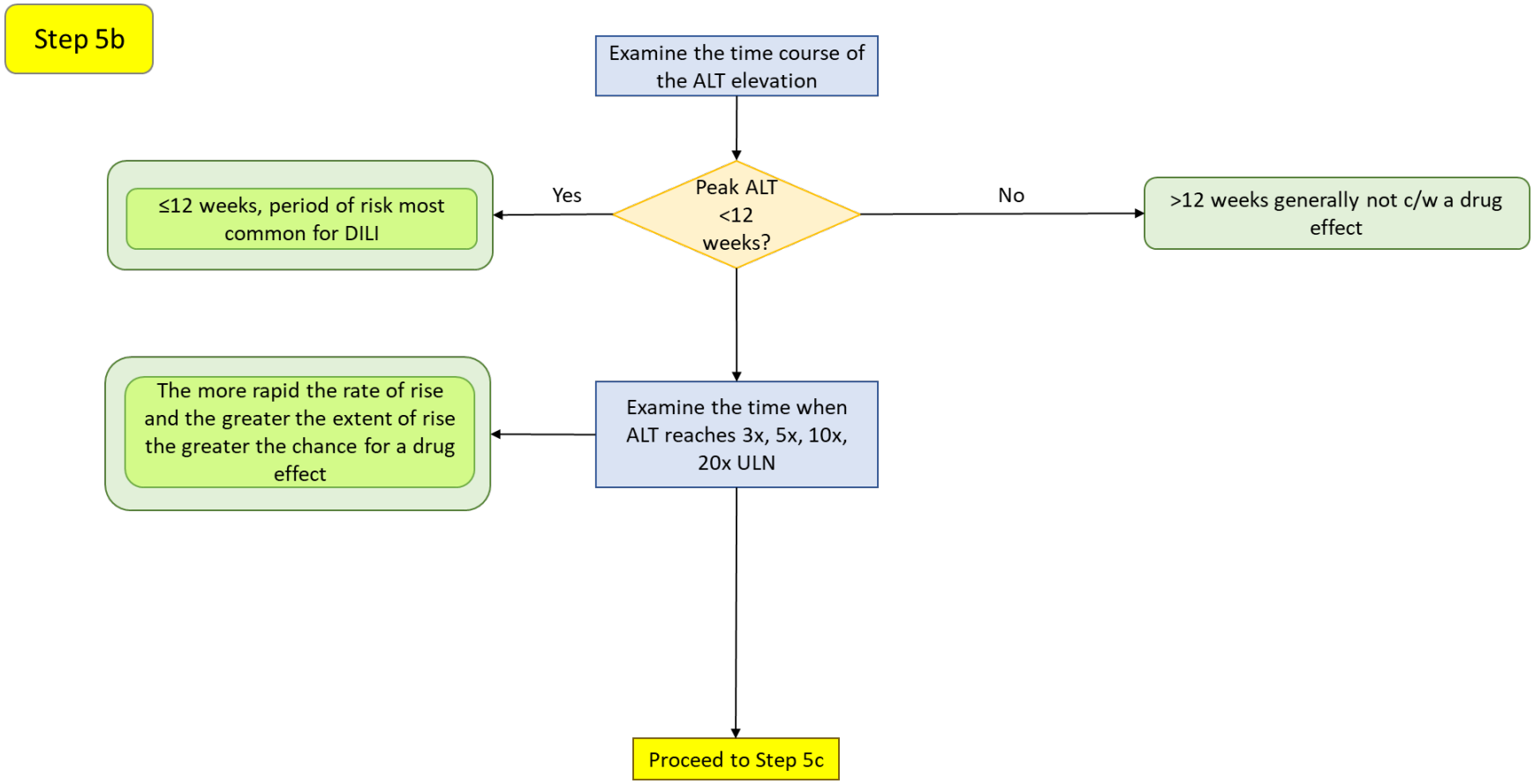

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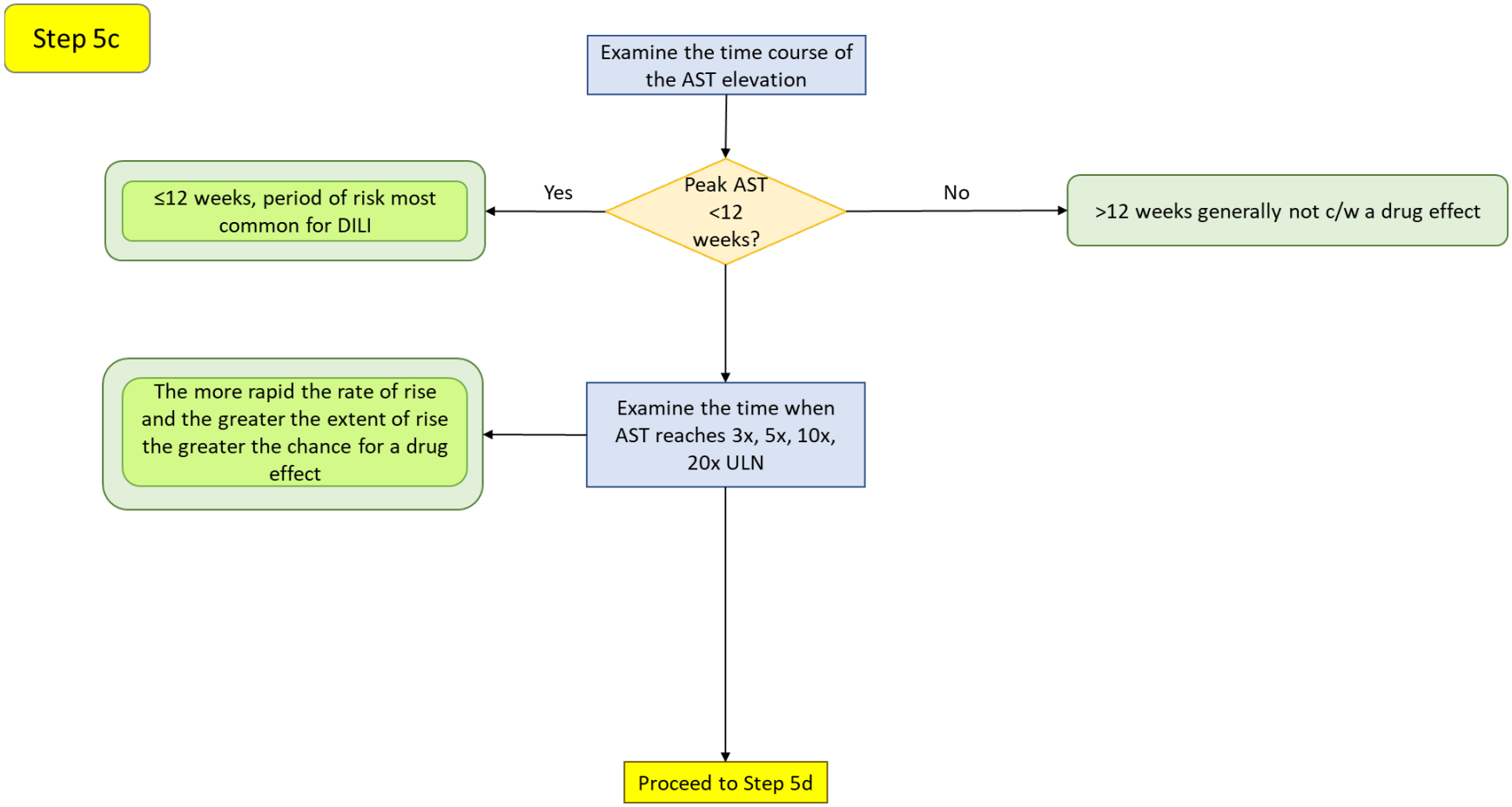

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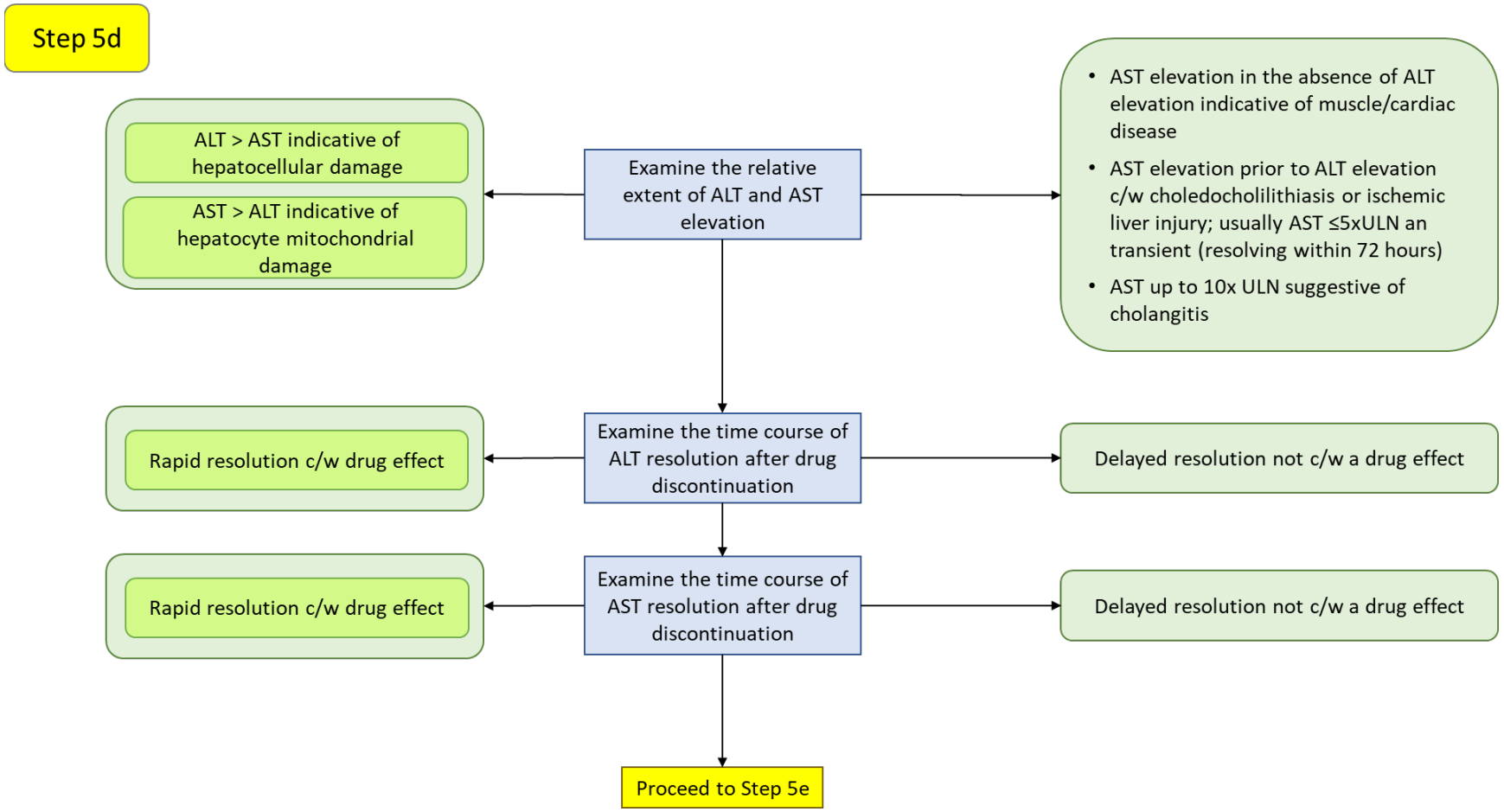
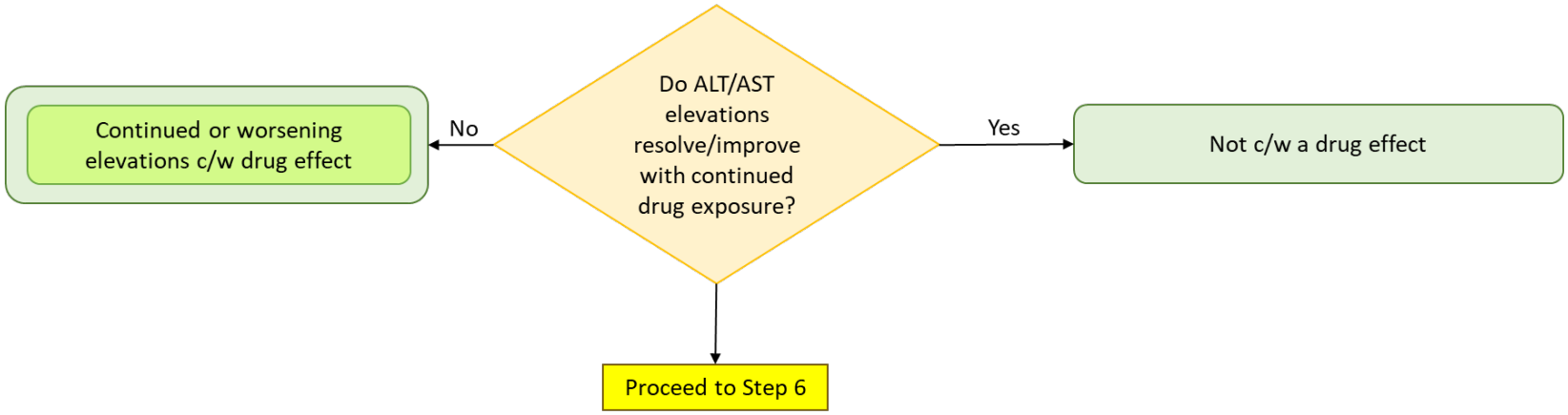


Interactive Safety Graphic – Hepatic Safety Explorer

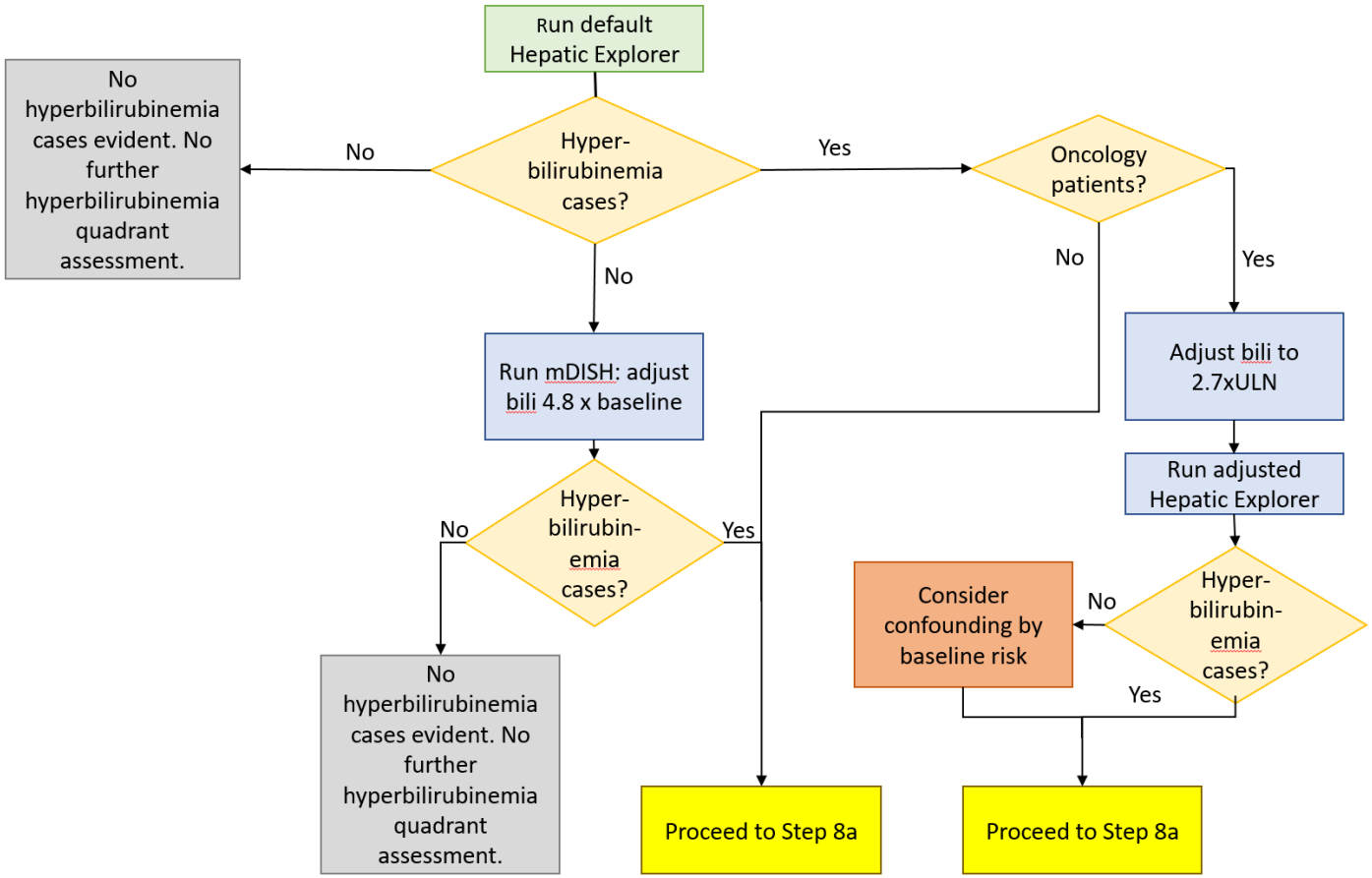
Step 5e



Note: Step 6 to be implemented in a future version.

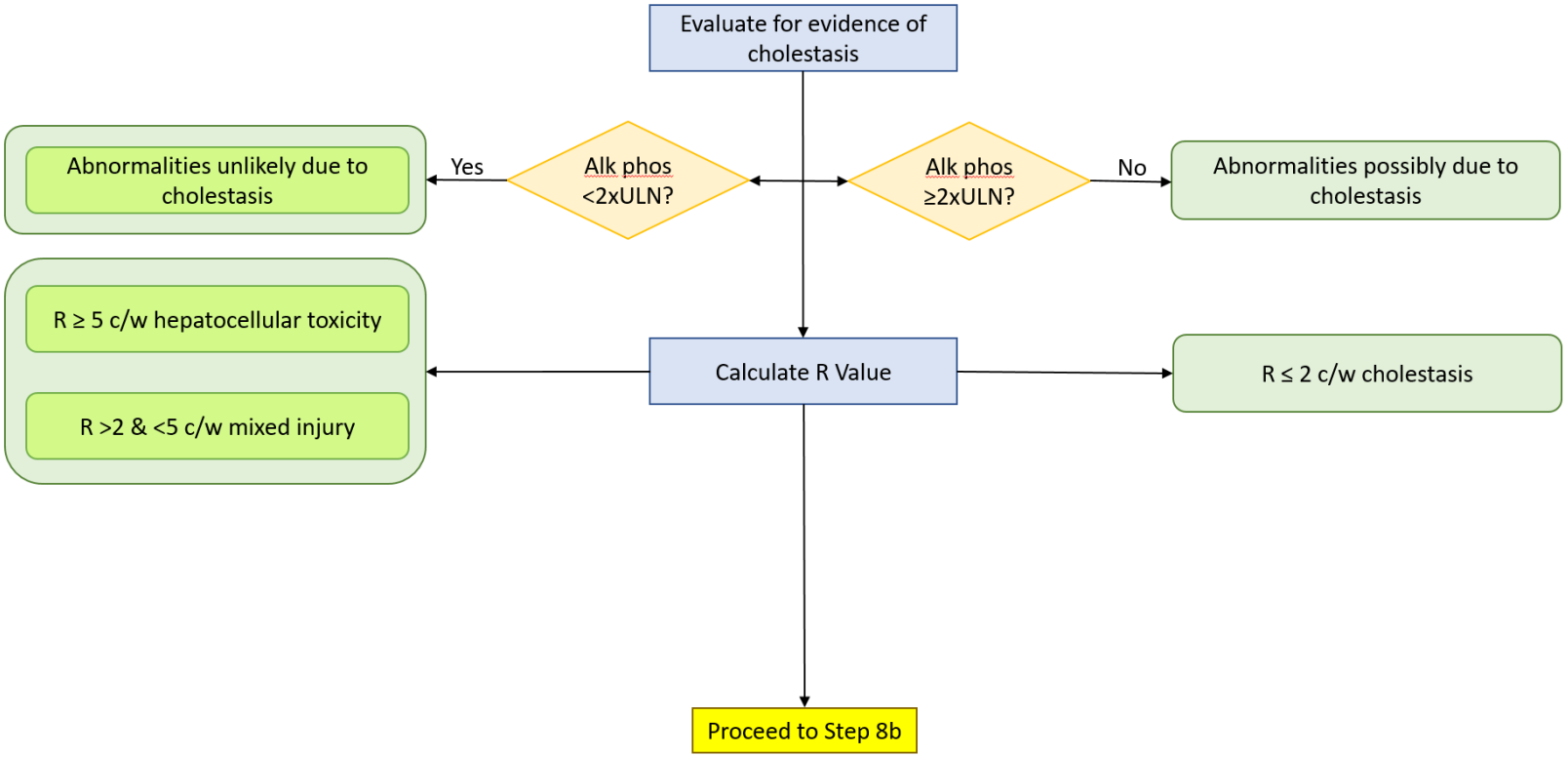
Appendix 3: Consolidated Hyperbilirubinemia Case Workflow

Step 7



Interactive Safety Graphic – Hepatic Safety Explorer

Step 8a



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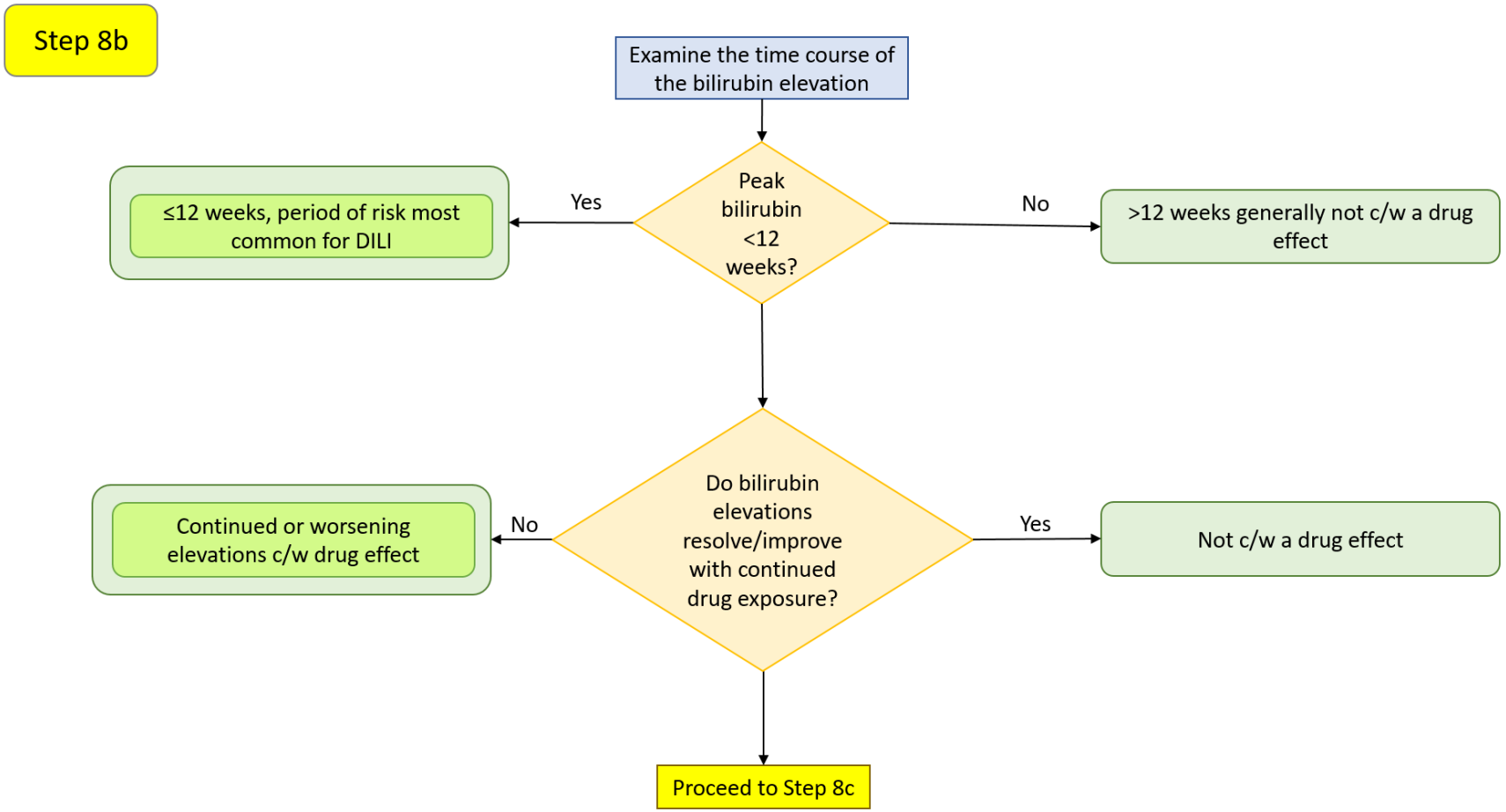

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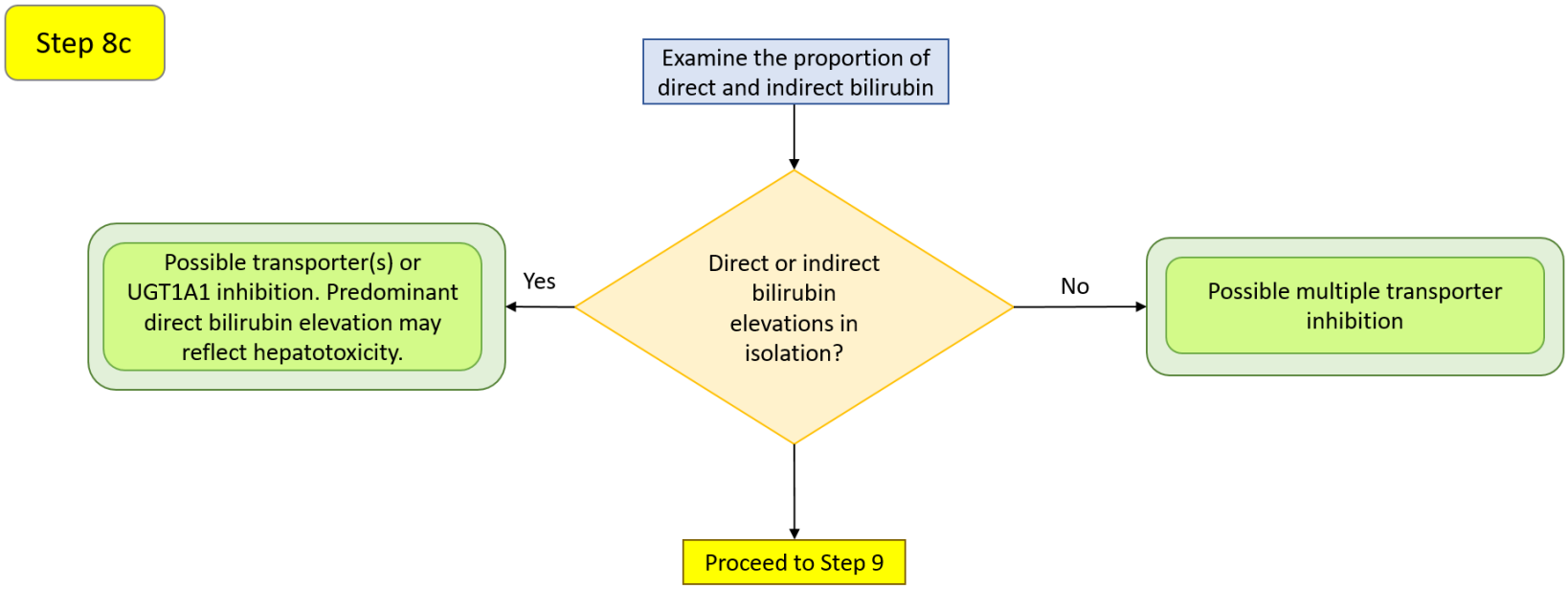

Note: Step 9 to be implemented in a future version.

Appendix 4: Abbreviated Potential Hy's Law Case Evaluation

Hy's Law cases?	**Yes**: The appearance of cases in the potential Hy's Law quadrant could represent drug-induced liver injury or could be the result of underlying risk factors or other confounders.
Peak ALT and bilirubin within 4 weeks?	**Yes**: Only elevations of bilirubin coincident with or shortly after peak ALT elevations are indicative of loss of hepatic function associated with liver injury (Merz et al. 2014, Longo et al. 2017). While there is no defined interval as a standard, peaks within 2 weeks are suggestive of DILI and up to 4 weeks may still indicate a drug effect. This can be assessed in the tool by defining the interval in the **Highlight Points Based on Timing** field**.** Alternatively, this can be manually reviewed by clicking on the point of interest in the Hy's Law quadrant and review the individual ALT and total bilirubin graphs displayed below the graph for that patient.
	No: Peak ALT and bilirubin values exceeding 4 weeks apart are less indicative of a drug-induced cause. A peak total bilirubin level that precedes a peak ALT level is not a typical pattern for hepatocellular injury (Watkins et al. 2011).
	Note: A peak total bilirubin level that precedes a peak ALT level is not a typical pattern for hepatocellular injury (Watkins et al. 2011).
Qualifying ALT and bilirubin within 4 weeks?	A limitation of the graph is the use of peak values to define the position of cases within the graph. "Qualifying" values are those that exceed the ULN threshold for either ALT or total bilirubin, but don't represent peak values (Merz et al. 2012). **Yes**: Qualifying ALT and total bilirubin values occurring within 2 weeks of each other, and with the rise in bilirubin following the transaminase rise, are suggestive of DILI. While there is no defined standard interval, qualifying values within 4 weeks may still indicated a drug-related effect. **No**: Qualifying ALT elevations exceeding 4 weeks apart are less indicative of a drug-induced cause.
Alk phos <2 x ULN?	**Yes**: A component of the Hy's Law definition is the presence of transaminase and bilirubin elevation without initial findings of cholestasis (e.g., elevated alkaline phosphatase) (FDA 2009). The lack of an elevated alkaline phosphatase has been defined as <2x ULN (Avigan 2010). Transaminase and bilirubin elevations meeting Hy's Law criteria in the absence of a concomitant elevation of alkaline phosphatase is indicative of hepatocellular injury. **No**: An elevated alkaline phosphatase level coincident with transaminase and bilirubin elevations may indicate a cholestatic source

	of the bilirubin elevation which could discount drug-related hepatocellular damage. However, this does not remove the possibility of drug-related cholestatic injury. **Note**: Alkaline phosphatase can be elevated by infiltrative diseases of the liver, tumors of hepatic and non-hepatic origin and bone diseases, including metastases to bone (AGA Clinical Practice Committee 2002). In such circumstances, these factors confound the ability to assess whether bilirubin elevations may be due to a cholestatic process.
Case evaluation	If… 1. The elevation of ALT is >3xULN and the elevation of total bilirubin is >2xULN, and 2. These elevations occur within 4 weeks of each other, and 3. The ALT elevation precedes the bilirubin elevation, and 4. The alkaline phosphatase is <2xULN, Then… The case may represent a potential Hy's Law case and should be referred for more detailed evaluation.