ORIGINAL RESEARCH

Are Laboratory Parameter (Biomarker) Values Similar to the Healthy Volunteer Reference Range in Individuals with Diabetes

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Background: Identification of laboratory parameter clinical safety signals depends on the terminology and scoring criteria. Grade 1 scoring criteria in the Common Terminology Criteria for Adverse Events (CTCAE) is typically based on the healthy volunteer reference range (HVRR). The objectives of this study were to determine 1) what laboratory parameters in individuals with diabetes are potentially different from the HVRR and 2) what fold change from baseline should be expected in this population.

Materials and Methods: Baseline data from the individuals with diabetes clinical trial data (TransCelerate dataset) were compared to the HVRR using a 10% threshold above HVRR to classify laboratory parameters as potentially different from the HVRR. These parameters were then evaluated longitudinally to determine the expected x-baseline values for individuals with diabetes for potential use in identifying drug-induced changes.

Results: The baseline data determined that 28% of the laboratory parameters evaluated were potentially different from the HVRR. Longitudinal data analysis determined 1) thresholds for 13 of these laboratory parameters with the subjects above the threshold having greater variability than those below the threshold, and 2) the expected upper limits (x-baseline) were calculated for the laboratory parameters. For example, a 1.8–2.6 x-baseline value for alanine aminotransferase, depending on how the baseline is calculated, is expected in individuals with diabetes.

Conclusion: It is not uncommon for laboratory parameters in individuals with diabetes clinical trials to be potentially different from the HVRR, and the x-baseline criteria for 13 of these laboratory biomarkers was determined for this population. This suggests consideration in modifying the current CTCAE grade 1 criteria of >1.5–3.0 x-baseline should be further investigated as to if the current criteria detects too many false-positive signals in this population.

Keywords: diabetes, biomarkers, reference range, laboratory parameter, pharmacovigilance

Introduction

Identification of potential clinical safety signals (adverse event: AE) is imperative during the clinical development of investigational new drugs/molecules to ensure the safety of the individuals and to determine the risk–benefit balance of the molecule. Safety signals are typically recorded using Medical Dictionary for Regulatory Activities (MedDRA) terms with laboratory parameters under the System Organ Classification of "Investigational" and each AE is based on a laboratory parameter being the preferred term (PT). For example, activated partial

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thromboplastin time prolonged, alanine aminotransferase increased, cholesterol high and CPK increased are the PT AE terms for some laboratory parameters.

The severity score for the laboratory parameters is based on the Common Terminology Criteria for Adverse Event (CTCAE) or similar scoring criteria. The lowest CTCAE severity is grade 1 with the definition of "mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated". Table 1 shows the CTCAE grade 1 criteria differences for some of the laboratory parameters between version 4.03 and version 5.0.[1,2] In version 4.03 the grade 1 criteria were based solely on the healthy volunteer reference range (HVRR) upper limits of normal (ULN). However, these definitions were modified in CTCAE version 5.0 for the liver function laboratory parameters to also include fold change from baseline, if the baseline value (prior to subject getting the drug) was above the HVRR ULN. This change in the grade 1 criteria was to help identify potential clinical safety signals in individuals that had elevated liver function tests at baseline, such as patients with NASH or liver metastases. However, based on a pilot study recently reported, we know that liver function laboratory parameters are not the only laboratory parameters above the HVRR ULN at baseline, but rather approximately 25% of the laboratory parameters across various patient populations evaluated were above the HVRR ULN.[3]

Table 1 Common Terminology Criteria for Adverse Event (CTCAE Grade 1 Scoring Definitions)

Adverse Event	Grade 1 Grade Criteria	
	CTCAE v4.03[1]	CTCAE v5.0[2]
Activated partial thromboplastin time prolonged	>ULN - 1.5 × ULN	>1.5–2.5 × ULN
Alanine aminotransferase increased	>ULN - 3.0 × ULN	>ULN - 3.0 × ULN if baseline was normal; 1.5–3.0 × baseline if baseline was abnormal
Aspartate aminotransferase increased	>ULN - 3.0 × ULN	>ULN - 3.0 × ULN if baseline was normal; 1.5–3.0 × baseline if baseline was abnormal
Cholesterol high	>ULN - 300 mg/dL; >ULN - 7.75 mmol/L	>ULN - 300 mg/dL; >ULN - 7.75 mmol/L
CPK increased	>ULN - 2.5 × ULN	>ULN - 2.5 × ULN
Creatinine increased	>1–1.5 × baseline; >ULN - 1.5 × ULN	>ULN - 1.5 × ULN
Lipase	>ULN - 1.5 × ULN	>ULN - 1.5 × ULN

Based on general medical knowledge it is already known that liver function tests and pancreatic enzymes are abnormal in individuals with diabetes: alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, amylase, gamma-glutamyl transferase, and lipase are elevated in 9–18%, 3–10%, 3–9%, 3–12%, 6–19% and 11–20% of the individuals, respectively.[4–7]

Determining if a change in CTCAE grade 1 criteria was needed for laboratory parameters, in addition to the liver function parameters and pancreatic enzymes, a large dataset of longitudinal data points was necessary. The objective of this study was to use the individuals with diabetes dataset from the TransCelerate Placebo Standard of Care (PSoC) project to determine if laboratory parameters are potentially different from the HVRR and based on the within-subject variability, what fold change from baseline should be expected in PSoC individuals with diabetes.

Materials and Methods
Dataset
Individuals with diabetes data were selected from batch 6 of the TransCelerate BioPharma PSoC products. A full description of the PSoC dataset is contained in the White Paper published by the collaborative[8] Briefly, companies within the consortium contribute data from PSoC arms of their studies in the standard format. All subjects were deidentified. Institutional review board (IRB) or ethics committee review was done by each individual company before initiating their clinical trial and so no additional IRB or ethics committee review was necessary for this specific manuscript. All consortia member companies have access to the full dataset, but it is not freely accessible to the scientific community.

Therefore, the PSoC database incorporates a large volume of Good Clinical Practices (GCP)-compliant clinical trial data (comprised of both placebo and standard of care) to facilitate the development of innovative drug products. There were 15 clinical trials in the individuals with diabetes TransCelerate database, batch 6 (Table 2). The protocols for each clinical trial were reviewed in ClinTrials.gov to ensure only data from similar populations were evaluated. Study 000001 consisted of patients with anemia in chronic kidney disease and study 12450048 were individuals with diabetes and hypertensive disorder and were thereby excluded from further analysis. In addition, the data from study 12180036 were excluded from further analysis due to technical issues. The total number of subjects analyzed with laboratory parameter values were 2825.

Table 2 Studies

Study Population	Study ID	Subject Number	Title	Study Duration
Anemia in chronic kidney disease	000001	2026		
Type 2 diabetes mellitus	12180015	130	A Randomised, Double-Blind, Placebo Controlled, Parallel Group 24 Week Study to Assess the Efficacy and Safety of BI 1356 (5 mg) in Combination With 30 mg Pioglitazone (Both Administered Orally Once Daily), Compared to 30 mg Pioglitazone Plus Placebo in Drug Naive or Previously Treated Type 2 Diabetic Patients with Insufficient Glycaemic Control	24 weeks
Type 2 diabetes mellitus	12180016	167	A Randomised, Double-Blind, Placebo-Controlled Parallel Group Efficacy and Safety Study of BI 1356 (5 mg Administered Orally Once Daily) Over 24 Weeks, in Drug Naive or Previously Treated (6 Weeks Washout) Type 2 Diabetic Patients with Insufficient Glycemic Control	24 weeks
Type 2 diabetes mellitus	12180017	177	A Randomised, Double-Blind, Placebo-Controlled Parallel Group Efficacy and Safety Study of BI 1356 (One Dose, eg 5 mg), Administered Orally Once Daily Over 24 Weeks, With an Open Label Extension to 80 Weeks (Placebo Patients Switched to BI 1356), in Type 2 Diabetic Patients with Insufficient Glycaemic Control Despite Metformin Therapy	80 weeks
Type 2 diabetes mellitus	12180018	263	A Randomised, Double-Blind, Placebo-Controlled Parallel Group Efficacy and Safety Study of BI 1356 (5 mg) Administered Orally Once Daily Over 24 Weeks, With an Open-Label Extension to One Year (Placebo Patients Switched to BI 1356), in Type 2 Diabetic Patients with Insufficient Glycaemic Control Despite a Therapy of Metformin in Combination with a Sulphonylurea	24 weeks
Type 2 diabetes mellitus	12180036 NCT00954447	627	A Phase III Randomised, double-blind, placebo-controlled, parallel group efficacy and safety study of linagliptin (5mg), administered orally once daily for at least 52 weeks in type 2 diabetic patients in combination with basal insulin therapy	52 weeks
Type 2 diabetes mellitus	12450019 NCT01210001	165	A randomized, double-blind, placebo controlled, parallel group efficacy and safety study of BI 10773 (10 and 25 mg administered orally once daily) over 24 weeks in type 2 diabetic patients with insufficient glycaemic control despite background therapy with Pioglitazone alone or in combination with metformin	24 weeks
Type 2 diabetes mellitus	12450023 NCT01159600	437	A Phase III randomized, double-blind, placebo-controlled, parallel group, efficacy and safety study of BI 10773 (10 mg, 25 mg) administered orally, once daily over 24 weeks in patients with type 2 diabetes mellitus with insufficient glycaemic control despite treatment with metformin alone on metformin in combination with a sulfonylurea	24 weeks
Type 2 diabetes mellitus	12450036 NCT01164501	319	A Phase III, randomized, double-blind, placebo-controlled, parallel group, efficacy and safety study of BI 10773 (10 mg and 25 mg administered once daily) as add on to pre-existing antidiabetic therapy over 52 weeks in patients with type 2 diabetes mellitus and renal impairment and insufficient glycaemic control C-SCADE-5	52 weeks
Type 2 diabetes mellitus:hypertensive disorder	12450048 NCT01370005	271	A Phase III randomized, double-blind, placebo-controlled, parallel group, efficacy and safety study of BI 10773 (10 mg, 25 mf) administered orally, once daily over 12 weeks in hypertensive patients with type 2 diabetes mellitus	12 weeks

(*Continued*)

Table 2 (Continued).

Study Population	Study ID	Subject Number	Title	Study Duration
Type 2 diabetes mellitus	D1680c00001	264	A 52-Week International, Multi-centre, Randomized, Parallel-group, Double-blind, Active-controlled, Phase III Study With a 52-Week Extension Period to Evaluate the Safety and Efficacy of Saxagliptin in Combination with Metformin Compared with Sulphonylurea in Combination with Metformin in Adult Patients with Type 2 Diabetes Who Have Inadequate Glycaemic Control on Metformin Therapy Alone	52 weeks
Type 2 diabetes mellitus	D1680c00005	258	A 24-Week International, Multi-Centre, Randomized, Parallel-Group, Double-Blind, Placebo-Controlled, Phase III Study to Evaluate the Efficacy and Safety of Saxagliptin in Adult Patients with Type 2 Diabetes Who Have Inadequate Glycaemic Control with Diet and Exercise	24 weeks
Type 2 diabetes mellitus	H9XMCGBDA	141	A Randomized, Placebo-Controlled Comparison of the Effects of Two Doses of LY2189265 or Exenatide on Glycemic Control in Patients with Type 2 Diabetes on Stable Doses of Metformin and Pioglitazone (AWARD-1: Assessment of Weekly Administration of LY2189265 in Diabetes-1)	52 weeks
Type 2 diabetes mellitus	H9XMCGBDC NCT01149421	268	The effect of LY2189265 on blood pressure and heart rate, as assessed by ambulatory blood pressure monitoring, in patients with type 2 diabetes mellitus	16 weeks
Type 2 diabetes mellitus	H9XMCGBDN NCT01126580	250	The impact of LY2189265 versus metformin on glycemic control in early type 2 diabetes mellitus (AWARD-3) assessment of weekly administration of LY2189265 in diabetes-3	52 weeks

Notes: All 15 studies within the database are listed in this table although only 12 studies were analyzed. Studies 000001, 12450048 and 12180036 were excluded from further analysis.

Baseline data was defined as the minimum laboratory day (lbdy) in a study data tabulation model (SDTM). If an individual had more than one value for a specific test on the baseline date, one of the values was randomly selected by assigning a random number from a uniform distribution and choosing the minimum random number.

Longitudinal data was all lbdy in the study tabulation model (SDTM) and all laboratory parameter values were analyzed for each subject.

Data Analysis

Data for this paper comes from two SDTM tables: LB (laboratory) and DM (demographics). The healthy volunteer reference ranges may be different within and between studies as they are determined at the laboratory doing the sample analysis. To eliminate the influence of different reference ranges, all laboratory values were normalized to the ULN by dividing the value by the ULN, on an individual sample basis. Most data processing was done in SAS Enterprise Guide Version 7.15 on the AZ SAS Grid installation.

Laboratory parameters with at least 400 subjects were analyzed and interpreted, based on the average number (between 300 and 500) of subjects recommended for a reference interval.[9]

A laboratory parameter in a population was deemed potentially different from the HVRR if greater than 10% of the laboratory values from the population were above the ULN. The 10% threshold is four times higher than the expected percentage (2.5%), based on the definition of HVRR and was selected as a clinically meaningful difference to minimize the risk of false positives (classifying a laboratory parameter as different from the HVRR).

Longitudinal data analysis was based on the normalization of the data at each time point to the first (baseline) value for that subject and reported as x-baseline. An estimate of the maximum x-baseline expected for PSoC individuals with diabetes was determined using the following formula with the 95%upper limits (UL) of coefficient of variation (CV) being the mean within-subject percent coefficient of variation plus two times the standard error of the mean: (baseline value + (baseline values * 95%UL of STD * z))/baseline with z = 1.96. This formula assumed

Table 3 Demographics of the Subject in the Twelve Studies

Study	Age (yr; Mean)	% Female	% Asian	% Black or African American	% Multiple	% Other	% White	% Race Not Specified
12180015	ND	35	24.6	0.0	0.0	0.8	74.6	0.0
12180016	ND	53	45.5	0.0	0.0	0.0	53.9	0.6
12180017	ND	43	17.5	0.0	0.0	1.1	78.0	3.4
12180018	ND	52	53.2	0.4	0.0	0.4	43.3	2.7
12450019	ND	55	0.0	0.0	0.0	59.4	35.2	5.5
12450023	ND	47	0.0	0.0	0.0	52.2	46.0	1.8
12450036	ND	43	36.4	1.3	0.0	0.0	58.3	4.1
D1680C00001	ND	39	22.0	0.0	0.0	0.0	78.0	0.0
D1680C00005	ND	46	100.0	0.0	0.0	0.0	0.0	0.0
H9XMCGBDA	54.58	41	4.3	7.1	1.4	14.2	73.0	0.0
H9XMCGBDC	55.28	55	7.5	5.2	1.5	10.8	75.0	0.0
H9XMCGBDN	56.48	48	9.2	8.8	0.4	0.4	81.2	0.0

Notes: ND – not determined, the information was not in the database. Other = "American Indian or Alaska Native", "Native Hawaiian or other Pacific Islander" or "Non-White".

the baseline value was roughly the mean for the subject, which would be accurate if using multiple values for determining the baseline. However, when only one baseline value was measured, this baseline value could be the minimal expected value and so for single baseline measurements the following formula was used to determine the x-baseline upper limit: (baseline value + (baseline value * 95%UL of CV * 1.96 * 2))/baseline, since the minimum value would be two standard deviations below the actual mean value.

Results
Studies

The distribution of sample numbers across the remaining studies ranged from 130 to 437 individuals in each study. Therefore, it is unlikely that data in one clinical trial should have a disproportionate impact on the final data interpretation.

Demographics

Demographics of the 12 studies being evaluated from the individuals with diabetes dataset shows some differences between studies (Table 3). Subject age cannot be evaluated as this demographic is missing from most studies, probably due to the method used by companies for de-identification. As expected, individuals with diabetes were roughly 50% female (35% to 55%). Of interest, the ethnicity varied greatly between the studies: most studies were majority white (n=6 studies), two studies were split between white and other, and four studies had higher levels of Asian (36% to 100%).

Baseline

All of the laboratory parameters evaluated for each of the twelve studies are listed in Tables 4 and 5. The results for the laboratory parameters with baseline values in less than 400 subjects or any results evaluated in just one study are shown in Table 4 but due to the limited datapoints were not interpreted as to a laboratory parameter being potentially different from the HVRR.

The list and results of the laboratory parameters evaluated in at least 400 individuals and in at least two studies are in Table 5. As expected, the glucose levels in most individuals with diabetes (72.5%) were above the HVRR ULN. In total, approximately 28% of these laboratory parameters (Glucose along with 17 others) were potentially different from the HVRR.

Longitudinal

All laboratory parameters that were evaluated in at least 400 subjects, at a minimum of two timepoints per subject and in at least two studies were evaluated longitudinally to determine the number of laboratory values (tests) that were above the HVRR ULN (Table 6). There were 32 laboratory parameters in which none of the studies had more than 10% of the values above the HVRR ULN (Table 6 footnote). The other 32 laboratory parameters evaluated had greater than 10% of the tests above the HVRR ULN in at least one of the studies. Evaluating the total tests, approximately 33% (21 out of 64) of the laboratory parameters were potentially different from the HVRR.

The 21 laboratory parameters that were potentially different from the HVRR based on longitudinal analysis

Table 4 Laboratory Parameters Reported in <400 Subjects or in Only One Study

Laboratory Parameter		Number of Studies	Number of Subjects with a Baseline Value	% of Subjects with Value >ULN at Baseline
Category (LBCAT)	Test (LBTEST)			
Chemistry	Albumin/Creatinine	1	259	24.3*
Chemistry	Aldosterone	1	236	20.8*
Chemistry	Brain Natriuretic Peptide	1	105	24.8*
Chemistry	C Reactive Protein	1	241	48.1*
Chemistry	Calcitonin	3	239	0.8
Chemistry	Cholesterol/HDL-Cholesterol	1	246	23.6*
Chemistry	Creatine Kinase MB	7	193	25.4*
Chemistry	Cystatin C	1	125	3.2
Chemistry	Indirect Bilirubin	3	12	75.0*
Chemistry	Insulin, Total HL7	2	330	27.6*
Chemistry	Insulin, Total HM6	1	29	48.3*
Chemistry	Insulin, Total L17	2	138	32.6*
Chemistry	Metanephrine	1	72	0
Chemistry	N-Acetyl Glucosamide/Creatinine	1	124	79.8*
Chemistry	Non-HDL Cholesterol	1	246	0
Chemistry	Normetanephrine	1	216	2.8
Chemistry	Renin Activity	1	119	45.4*
Chemistry	Sex Hormone Binding Globulin	1	1	0
Chemistry	Testosterone	1	2	0
Chemistry	Thyrotropin	4	22	13.6*
Chemistry	Troponin I	2	5	100*
Hematology	Neutrophils Band Form	1	1	0
Hematology	Reticulocytes/Erythrocytes	3	184	2.7
Serology	Anti-Exenatide (Ly2148568) Antibodies	1	4	0
Urinalysis	Alpha-1 Microglobulin	2	135	27.4*
Urinalysis	Alpha-1 Microglobulin/Creatinine	2	134	30.6*

Notes: *The baseline value greater than 10% of the individuals with diabetes was higher than the healthy volunteer reference range (HVRR) upper limits of normal (ULN).

were plotted to determine if there was a difference in the natural variability between tests/subjects that were above the HVRR ULN versus those that were not (Figure 1). Thirteen of the 21 had a cutoff that was visibly distinguishable: Alanine aminotransferase = 1.5xULN, C-peptide = 1.5xULN, chemistry Creatinine =1.0xULN, Gamma Glutamyl Transferase = 1.5xULN, Glucagon = 1.0xULN, Lipase = 1.5xULN, Proinsulin = 2.0xULN, Triglycerides = 1.5xULN, Urate = 1.2xULN, Insulin = 2.0xULN, Hemoglobin A1C/Hemoglobin = 1.0xULN, Albumin = 10.0xULN, and Urea = 1.5x ULN. Cutoffs could not be determined for the other eight laboratory parameters.

The mean within-subject variability as measured by the percent coefficient of variation (%CV) was calculated for the 13 laboratory parameters that had a cutoff. It was calculated per study and for the total population in the twelve evaluated studies (Table 7). For all laboratory parameters, the within-subject variability was higher in the group above the cutoff than in the group below the cutoff.

The upper 95% confidence interval (95% CI) limit of the within-subject variability from the total population was used to calculate the upper limit of the expected (based on PSoC within-subject variability) x-baseline using a 95% CI. These values are in the column labeled "baseline = mean", since the formula used calculates the 95% CI from the mean (Tables 8 and 9). However, it is never known if the baseline value is the subjects mean value or if it could be the lowest expected value for that subject. Therefore, the column labeled baseline = minimum is the maximum potential x-baseline that should be observed for a subject (based on the 95% CI).

Discussion

The Objectives of this study were to determine 1) what laboratory parameters in individuals with diabetes were

Table 5 Laboratory Parameters Reported in >400 Subjects and in at Least Two Studies

Laboratory Parameter		Number of Studies	Number of Subjects with a Baseline Value	% of Subjects with Value >ULN at Baseline
Category (LBCAT)	Test (LBTEST)			
Chemistry	Alanine Aminotransferase	12	2825	15.5*
Chemistry	Albumin	12	2825	3.3
Chemistry	Alkaline Phosphatase	12	2825	7.2
Chemistry	Amylase	7	1390	6.3
Chemistry	Amylase, Pancreatic	3	653	4.6
Chemistry	Apolipoprotein A1	3	907	0.8
Chemistry	Apolipoprotein B	3	907	8.5
Chemistry	Aspartate Aminotransferase	12	2825	6.4
Chemistry	Bicarbonate	3	921	8.0
Chemistry	Bilirubin	12	2825	2.3
Chemistry	Blood Urea Nitrogen	4	903	3.3
Chemistry	C-peptide	4	904	6.4
Chemistry	Calcium	10	2303	5.5
Chemistry	Chloride	5	1443	0.9
Chemistry	Cholesterol	12	2783	22.4*
Chemistry	Creatine Kinase	12	2825	10.3*
Chemistry	Creatinine	12	2825	8.6
Chemistry	Creatinine Clearance	5	1167	48.8*
Chemistry	Direct Bilirubin	6	632	0.8
Chemistry	Free Fatty Acid	3	897	8.5
Chemistry	Gamma Glutamyl Transferase	10	1447	17.8*
Chemistry	Glomerular Filtration Rate	3	921	0
Chemistry	Glucagon	3	768	5.1
Chemistry	Glucose	9	1912	72.5*
Chemistry	HDL Cholesterol	12	2780	1.3
Chemistry	Insulin	4	880	21.8*
Chemistry	LDL Cholesterol	12	2762	14.5*
Chemistry	Lactate Dehydrogenase	7	1653	4.8
Chemistry	Lipase	6	1574	16.8*
Chemistry	Magnesium	3	921	0.3
Chemistry	N-Terminal ProB-type Natriuretic Peptide	3	428	20.3*
Chemistry	Phosphate	7	1658	3.3
Chemistry	Potassium	12	2824	3.5
Chemistry	Proinsulin	3	548	25.9*
Chemistry	Protein	9	2180	3.4
Chemistry	Sodium	12	2825	0.6
Chemistry	Triglycerides	12	2795	24.3*
Chemistry	Urate	10	2303	12.3*
Chemistry	Urea	6	1259	7.4
Hematology	Basophils	12	2803	0.1
Hematology	Basophils/Leukocytes	5	1442	0
Hematology	Eosinophils	12	2803	4.5
Hematology	Eosinophils/Leukocytes	5	1442	9.6
Hematology	Erythrocyte Mean Corpuscular HGB Concentration	3	621	0

(*Continued*)

Table 5 (Continued).

Laboratory Parameter		Number of Studies	Number of Subjects with a Baseline Value	% of Subjects with Value >ULN at Baseline
Category (LBCAT)	Test (LBTEST)			
Hematology	Erythrocyte Mean Corpuscular Volume	3	621	10.3*
Hematology	Erythrocytes	12	2805	1.5
Hematology	Hematocrit	12	2798	2.3
Hematology	Hemoglobin	12	2805	1.2
Hematology	Hemoglobin A1C	3	921	0
Hematology	Hemoglobin A1C/Hemoglobin	8	2097	56.1*
Hematology	Leukocytes	12	2805	2.8
Hematology	Lymphocytes	12	2803	3.7
Hematology	Lymphocytes/Leukocytes	5	1442	2.2
Hematology	Monocytes	12	2803	1.2
Hematology	Monocytes/Leukocytes	5	1442	0.8
Hematology	Neutrophils	9	2179	2.3
Hematology	Neutrophils, Segmented	3	624	1.4
Hematology	Neutrophils/Leukocytes	5	1442	2.0
Hematology	Platelets	12	2789	0.9
Urinalysis	Albumin	6	1431	28.4*
Urinalysis	Albumin/Creatinine	11	2378	30.8*
Urinalysis	Creatinine	3	634	0
Urinalysis	Urea	3	921	19.4*
Urinalysis	pH	5	1439	0.1

Notes: *Laboratory parameters deemed potentially different from the healthy volunteer reference range (HVRR), based on the baseline value greater than 10% of the individuals with diabetes being higher than the HVRR upper limits of normal (ULN).

potentially different from the HVRR and 2) what x-baseline should be expected in PSoC individuals with diabetes.

The TransCelerate database consisting of PSoC clinical trial data from across pharmaceutical companies contained 15 studies in the individuals with diabetes dataset, but only 12 of the studies consisting of approximately 2800 subjects had similar individual definitions and were further analyzed. The demographics did vary between studies with some being mainly white while one was 100% Asian, but no obvious demographic influences were observed within the data analysis.

Of the 64 laboratory parameters with baseline data from at least 400 subjects approximately 28% where considered potentially different from the HVRR, based on the definition of more than 10% of the subject baseline values being above the HVRR ULN. This 10% threshold was chosen to decrease the chance of false-positive classification of a laboratory parameter being different as a clinically meaningful difference from the HVRR without doing statistical analysis within this study. The HVRR

reference range is a 95% CI, meaning 2.5% of the values will be above the ULN and 2.5% of values will be below the lower limits of normal (LLN). The 10% cutoff is four times the expected 2.5%. The 28% of laboratory parameters being potentially different from the HVRR is very similar to the 25% observed across patient populations and the 25% observed in the individuals with diabetes population in a pilot study evaluating only 6 laboratory parameters.[3] Of the six parameters evaluated in the pilot study and this study, the results were very similar: alanine aminotransferase and glucose were potentially different from the HVRR, while neutrophil, platelets, aspartate aminotransferase and bilirubin were similar to the HVRR. Based on general medical knowledge and literature it is known that individuals with diabetes have abnormal levels of alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, amylase, gamma-glutamyl transferase, and lipase in 9–18%, 3–10%, 3–9%, 3–12%, 6–19% and 11–20% of the individuals, respectively.[4–7] This study also had abnormal levels in these same parameters that fell

Table 6 Percentage of Laboratory Values Above the Upper Limits of Normal, by Study (ULN)

	Laboratory Parameter	I2180015	I2180016	I2180017	I2180018	I2450019	I2450023	I2450036	D1680C00001	D1680C00005	H9XMCGBDA	H9XMCGBDC	H9XMCGBDN	Total
Chemistry	Alanine Aminotransferase	12.0*	13.0*	9.7	12.9*	8.0	15.0*	7.7	18.1*	8.7	10.7*	18.2*	17.2*	12.6*
Chemistry	Alkaline Phosphatase	2.8	2.6	3.0	3.8	6.3	5.3	11.1*	5.4	12.4*	5.1	7.6	6.4	6.9
Chemistry	Amylase	6.7	8.5	10.9*	13.7*	NT	TN	NT	NT	NT	6.9	1.7	3.5	6.6
Chemistry	Apolipoprotein B	NT	NT	NT	NT	17.3*	9.7	7.6	NT	NT	NT	NT	NT	9.9
Chemistry	C-peptide	NT	NT	NT	NT	NT	NT	NT	26.4*	10.0*	1.5	25.8*	NT	24.9*
Chemistry	Cholesterol	16.5*	20.0*	5.4	9.6	38.0*	23.8*	20.3*	25.5*	39.4*	24.5*	31.0*	19.6*	21.6*
Chemistry	Creatine Kinase	12.7*	10.8*	8.7	12.7*	10.4*	6.3	21.3*	13.0*	8.2	10.6*	10.1*	10.3*	11.8*
Chemistry	Creatinine	4.0	3.2	2.7	4.2	3.6	3.1	54.7*	2.2	0.9	3.2	0.8	4.8	10.9*
Chemistry	Creatinine Clearance	NT	NT	NT	NT	NT	NT	NT	62.9*	33.5*	44.8*	48.2*	45.2*	47.7*
Chemistry	Direct Bilirubin	NT	NT	NT	NT	NT	15.0*	54.5*	NT	NT	1.4	0.3	0.4	1.0
Chemistry	Free Fatty Acid	NT	NT	NT	NT	4.1	10.0*	6.2*	NT	NT	NT	NT	0.0	7.4
Chemistry	Gamma Glutamyl Transferase	14.5*	17.9*	7.7	12.4*	NT	28.3*	32.3*	NT	NT	13.2*	21.2*	18.5*	15.9*
Chemistry	Glucagon	NT	NT	NT	NT	NT	NT	NT	2.4	0.2	NT	14.9*	NT	12.6*
Chemistry	Glucose	96.8*	95.6*	95.6*	92.0*	NT	NT	NT	78.0*	90.1*	81.2*	50.8*	75.2*	78.8*
Chemistry	Insulin	NT	NT	NT	NT	NT	NT	NT	34.6*	30.8*	7.3	56.4*	NT	51.6*
Chemistry	LDL Cholesterol	14.8*	16.6*	3.6	5.6	25.2*	14.6*	12.7*	16.4*	26.4*	17.6*	16.4*	13.0*	14.1*
Chemistry	Lactate Dehydrogenase	3.3	9.5	3.1	6.6	4.1	1.7	10.1*	NT	NT	NT	NT	NT	6.1
Chemistry	Lipase	NT	NT	NT	NT	12.2*	14.2*	30.1*	NT	NT	21.0*	10.8*	13.4*	17.8*
Chemistry	N-Terminal ProB-type Natriuretic Peptide	NT	NT	NT	NT	24.6*	NT	NT	NT	NT	25.5*	NT	15.6*	21.3*
Chemistry	Potassium	7.1	8.4	8.9	10.2*	1.1	0.6	3.4	1.5	0.9	0.5	0.4	0.8	3.2
Chemistry	Proinsulin	NT	NT	NT	NT	NT	NT	NT	3.0	0.6	NT	40.6*	NT	21.1*
Chemistry	Protein	3.9	13.4*	7.3	6.1	2.0	0.6	1.0	0.3	1.8	NT	NT	NT	2.9
Chemistry	Triglycerides	47.5*	54.7*	52.6*	47.3*	9.8	14.4*	13.4*	15.3*	17.8*	13.4*	17.6*	15.3*	20.8*
Chemistry	Urate	8.5	10.7*	12.7*	10.3*	5.9	8.2	31.1*	NT	NT	5.6	12.4*	8.4	14.0*
Chemistry	Urea	16.5*	5.5	11.2*	15.6*	0.0	0.0	0.0	4.9	1.5	NT	NT	NT	7.2
Hematology	Eosinophils	3.0	5.9	5.8	2.4	10.2*	2.6	7.3	5.6	6.4	NT	2.9	3.0	5.1
Hematology	Eosinophils/Leukocytes	NT	NT	NT	NT	18.6*	5.5	11.1*	8.7	10.4*	NT	TN	NT	9.8
Hematology	Erythrocyte Mean Corpuscular Volume	NT	NT	NT	NT	NT	NT	NT	NT	NT	11.0*	11.3*	6.9	10.0*

(Continued)

Table 6 (Continued).

Laboratory Parameter		12180015	12180016	12180017	12180018	12450019	12450023	12450036	D1680C00001	D1680C00005	H9XMCGBDA	H9XMCGBDC	H9XMCGBDN	Total
Hematology	Hemoglobin A1C/Hemoglobin	NT	NT	NT	NT	0.0	0.0	0.0	88.2*	97.8*	98.1*	92.6*	99.6*	63.7*
Urinalysis	Albumin	NT	NT	NT	NT	36.0*	37.3*	65.2*	NT	NT	NT	NT	NT	38.8*
Urinalysis	Albumin/Creatinine	38.8*	27.7*	27.4*	30.2*	30.0*	25.5*	57.1*	NT	32.1*	24.5*	21.8*	26.1*	34.7*
Urinalysis	Urea	NT	NT	NT	NT	5.0	5.5	44.1*	NT	NT	NT	NT	NT	24.2*

Notes: The following laboratory parameters had less than 10.0% of laboratory tests above the HVRR ULN in all studies evaluated: Chemistry: albumin, amylase pancreatic, apolipoprotein A1, aspartate aminorransferase, bicarbonate, bilirubin, blood urea nitrogen, calcium, chloride, glomerular filtration rate, HDL cholesterol, magnesium, phosphate, sodium; Hematology: basophils, basophils/leukocytes, erythrocyte, Mean corpuscular concentration, erythrocytes, hematocrit, hemoglobin, hemoglobin A1C, leukocytes, lymphocytes, lymphocytes/leukocytes, monocytes, monocytes/leukocytes, neutrophils, neutrophils-segmented, neutrophils/leukocytes, platelets; urinalysis: creatinine, pH. NT Laboratory parameter was not evaluated in each subject at least two times during the study. *Greater than 10% of tests were higher than the upper limits of normal (ULN).

within the expected ranges with alanine aminotransferase, gamma-glutamyl transferase and lipase all above the 10% threshold to be potentially different from the HVRR. The other parameters aspartate aminotransferase, alkaline phosphate and amylase although elevated as expected in some of the individuals with diabetes the levels were less than 10%. This suggests that the 10% threshold we have selected if anything may be too high in detecting laboratory biomarkers that are known to be elevated in a patient population. Only two of the hematology laboratory parameters were determined to be potentially different from the HVRR: as expected hemoglobin A1C/hemoglobin ratio but unexpectedly erythrocyte mean corpuscular volume. It has been shown that the erythrocyte mean corpuscular volume and some other erythrocyte laboratory parameters can be elevated in individuals with diabetes.[10,11] However, these abnormal values are thought to be due to the elevated glucose levels in the cell inducing a hyperosmolar swelling of the erythrocytes and not a permanent homeostatic change to the cells. Of the laboratory parameters with less than the required 400 baseline values, it is of interest that brain natriuretic peptide, C-reactive protein and urinary alpha-1 microglobulin were greater than the ULN in more than 10% of the subjects as according to literature these three laboratory biomarkers have been shown to be elevated in individuals with diabetes.[12,13] This data, although with less than 400 subjects, may, therefore, be useful in better understanding the more currently esoteric laboratory parameters.

The 28% of laboratory parameters being potentially different from the HVRR further support the pilot study and general medical judgement that it is not uncommon for laboratory parameter values to be different from the HVRR in patient populations. Furthermore, it drives the need for additional analysis of patient population data to determine if the strategy for detecting potential drug-induced signals needs to be modified. Such as the CTCAE grade 1 scoring definitions being modified for laboratory parameters, similar to the definition changes for liver function tests between CTCAE v4.03 and v5.0.

To initiate this additional analysis, the TransCelerate individuals with diabetes dataset was evaluated longitudinally with 21 of the laboratory parameters being potentially different from the HVRR. There were at least two different approaches to elucidate the expected laboratory parameter variability: use the grade 1 CTCAE cutoffs of 1.0xULN or determine if another cutoff should be used based on the data. Using the 1.0xULN cutoff could underestimate the

laboratory parameter expected values as values in all subjects in a population could be shifted toward the ULN and thereby leading researchers to conclude that elevations seen during clinical trials might be a drug effect as opposed to natural disease progression. On the other hand, too high of a cutoff could decrease the number of subjects analyzed such that the result would be meaningless for signal identification/evaluation. The approach used in this study was to determine if there was an obvious threshold based on the data. Of the 21 laboratory parameters potentially different from the HVRR, 13 of them had an identifiable threshold. More data is needed to determine if such a cutoff can be made for the additional 8 laboratory parameters.

Using the 95% CI upper limit of the within-subject variability (%CV) the upper limits of the 95% CI were determined for each laboratory parameter and then reported as "x-baseline" with the baseline reported as xULN. These are the

values, with more data, that could be used for changing the CTCAE grade 1 definition and signal detection in individuals with diabetes clinical trials, but the formula assumes that the baseline value would be the mean for that subject, which would be more accurate if a mean baseline value was used based on two or more baseline values measured at different visits. However, some companies and studies use only one baseline value to evaluate/identify potential safety signals in that case the "baseline = minimum" values should be used. These values assume that the one baseline measure is the minimum value for the subject and therefore if a future measure for that value is greater than this x-baseline value then it is most likely not due to the natural laboratory parameter variability (based on the 95% CI).

Worth noting are the results for the liver enzyme data. According to the CTCAE v5.0, the grade 1 criteria is 1.5–3.0 x-baseline if the baseline is abnormal, but according to the
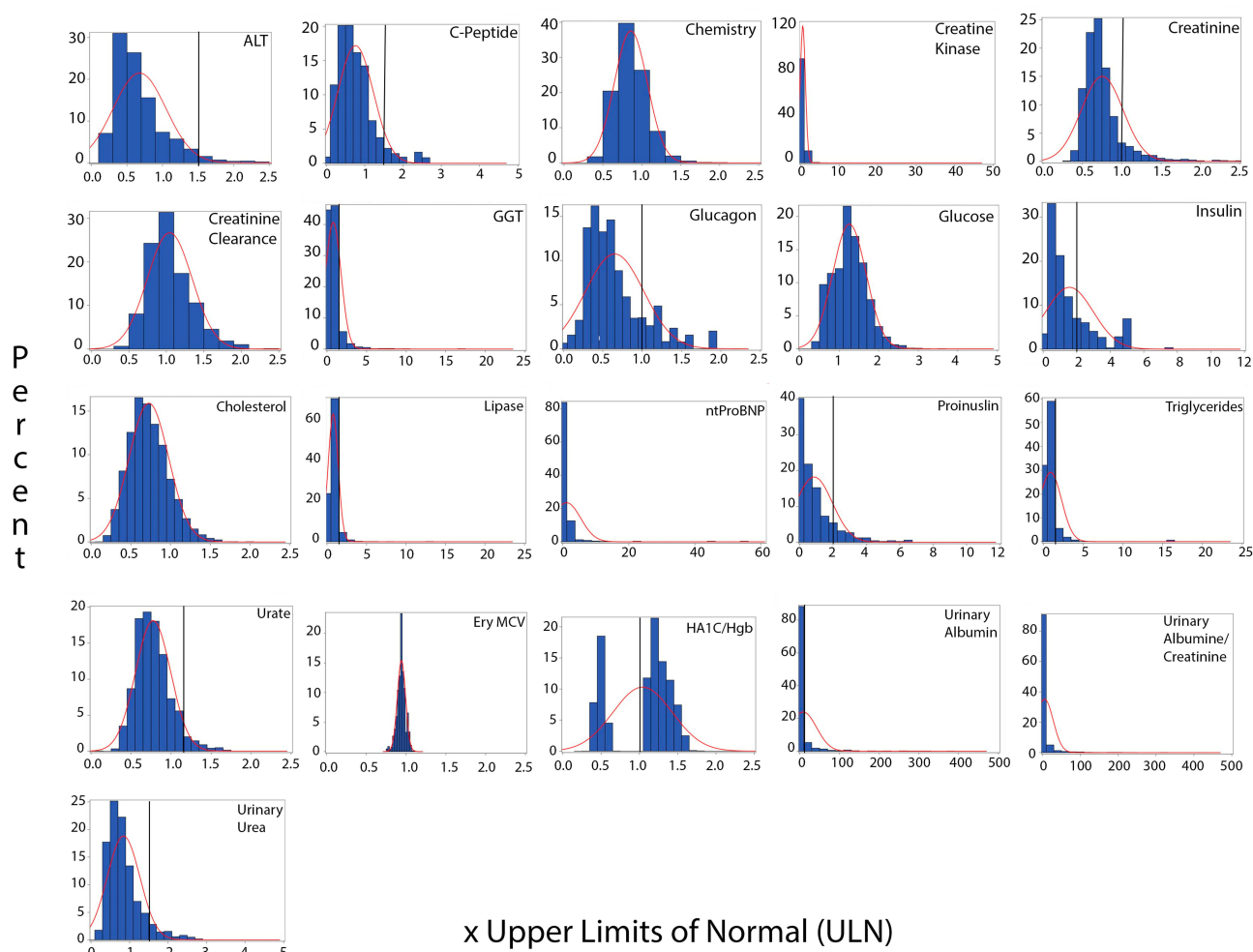
Figure 1 Longitudinal laboratory values (time upper limits of normal; xULN) of laboratory parameters that were potentially different from the healthy volunteer reference range. Red line is the curve fit calculation to the data and black vertical line is the determined threshold. Thresholds could not be determined for all laboratory parameters.

Table 7 Mean Within-Subject Variability (%CV)

Laboratory Parameter		Threshold (xULN)	12180015	12180016	12180017	12180018	12450019	12450023	12450036	D1680C 00001	D1680C 00005	H9XMCG BDA	H9XMCG BDC	H9XMCG BDN	Total
Chemistry	Alanine Aminotransferase	Total	21.3	22.1	21.2	21.1	20.7	20.2	21.1	21.5	24.6	25.1	24.5	19.2	21.7
		Below 1.5	20.3	20.5	19.9	20.1	19.6	18.8	19.4	19.2	22.9	23.7	21.9	18.3	20.2
		Above 1.5	38.8	40.2	38.1	29.5	44.6	32.9	43.4	35.9	50.8	45.7	38.0	28.2	37.0
Chemistry	c-peptide	Total	NT	NT	NT	NT	NT	NT	NT	20.7	33.1	18.4	33.1	NT	27.5
		Below 1.5	NT	NT	NT	NT	NT	NT	NT	18.3	32.1	18.4	30.2	NT	25.4
		Above 1.5	NT	NT	NT	NT	NT	NT	NT	52.8	51.1		44.6	NT	47.4
Chemistry	Creatinine	Total	6.9	7.4	8.8	7.7	8.4	7.0	8.7	8.6	7.9	7.6	7.4	7.7	7.8
		Below 1.0	6.6	6.6	8.3	7.4	7.8	6.8	7.0	7.2	7.3	6.5	7.1	7.3	7.1
		Above 1.0	11.3	16.7	16.0	10.5	15.2	10.8	9.5	23.3	24.9	21.4	20.2	10.1	12.1
Chemistry	Gamma Glutamyl Transferase	Total	21.4	19.0	15.7	18.0	NT	12.7	20.7	NT	NT	27.6	23.5	16.6	19.8
		Below 1.5	19.1	17.0	15.4	15.9	NT	10.4	19.8	NT	NT	25.1	20.2	15.0	17.8
		Above 1.5	37.6	29.1	22.1	36.1	NT	24.1	23.6	NT	NT	44.3	37.9	27.7	33.6
Chemistry	Glucagon	Total	NT	NT	NT	NT	NT	NT	NT	20.4	18.6	NT	28.1	NT	22.4
		Below 1.0	NT	NT	NT	NT	NT	NT	NT	19.8	18.5	NT	26.7	NT	21.1
		Above 1.0	NT	NT	NT	NT	NT	NT	NT	29.0	31.7	NT	31.0	NT	30.7
Chemistry	Insulin	Total	NT	NT	NT	NT	NT	NT	NT	29.5	35.0	26.1	51.0	NT	36.8
		Below 2.0	NT	NT	NT	NT	NT	NT	NT	26.4	32.5	25.6	32.9	NT	29.3
		Above 2.0	NT	NT	NT	NT	NT	NT	NT	63.0	54.9	89.5	69.3	NT	66.5
Chemistry	Lipase	Total	NT	NT	NT	NT	18.9	20.2	24.4	NT	NT	27.0	24.3	23.8	22.8
		Below 1.5	NT	NT	NT	NT	16.3	15.4	16.8	NT	NT	18.1	17.5	18.9	16.9
		Above 1.5	NT	NT	NT	NT	45.7	56.5	42.7	NT	NT	64.4	61.9	59.2	52.8
Chemistry	Proinsulin	Total	NT	NT	NT	NT	NT	NT	NT	34.9	28.4	NT	40.6	NT	34.5
		Below 2.0	NT	NT	NT	NT	NT	NT	NT	34.9	27.9	NT	39.2	NT	32.9
		Above 2.0	NT	NT	NT	NT	NT	NT	NT		78.5	NT	43.9	NT	44.9
Chemistry	Triglycerides	Total	24.7	23.0	23.1	24.5	21.8	22.1	21.6	23.1	21.4	22.4	20.6	19.9	22.2
		Below 1.5	19.9	17.8	18.9	19.2	21.1	21.1	20.5	21.0	19.4	21.4	18.0	18.0	19.9
		Above 1.5	32.6	31.5	29.0	33.8	48.4	35.3	35.1	41.0	39.2	31.9	37.4	42.4	34.2
Chemistry	Urate	Total	10.8	10.3	10.0	10.4	9.5	10.1	9.9	NT	NT	10.8	10.5	10.4	10.2
		Below 1.2	10.7	10.2	10.0	10.1	9.4	10.1	9.3	NT	NT	10.7	10.1	10.2	10.0
		Above 1.2	13.9	12.1	12.7	15.7	16.3	11.6	11.7	NT	NT	13.5	15.7	15.5	12.9
Hematology	Hemoglobin A1C/ Hemoglobin	Total	NT	NT	NT	NT	6.4	5.5	6.3	6.9	7.4	9.0	6.8	6.1	6.6
		Below 1.0	NT	NT	NT	NT	6.4	5.5	6.3						5.9
		Above 1.0	NT	NT	NT	NT				6.9	7.4	9.0	6.8	6.1	7.1
Urinalysis	Albumin	Total	NT	NT	NT	NT	61.2	55.8	63.0	NT	NT	NT	NT	NT	55.9
		Below 10.0	NT	NT	NT	NT	89.9	54.8	60.6	NT	NT	NT	NT	NT	54.4
		Above 10.0	NT	NT	NT	NT	78.5	66.1	67.0	NT	NT	NT	NT	NT	67.5
Urinalysis	Urea	Total	NT	NT	NT	NT	16.7	15.6	15.3	NT	NT	NT	NT	NT	15.7
		Below 1.5	NT	NT	NT	NT	16.7	15.6	14.3	NT	NT	NT	NT	NT	15.5
		Above 1.5	NT	NT	NT	NT	8.9	15.4	17.7	NT	NT	NT	NT	NT	17.5

Notes: NT laboratory parameter was not evaluated at least two times in subjects in this study. The following laboratory parameters did not have an obvious cutoff: cholesterol, creatine kinase, creatinine clearance, glucose, LDL cholesterol, n-terminal proB-type natriuretic peptide, erythrocyte. Mean corpuscular volume, and albumin/creatinine.

Table 8 Expected Fold Baseline, Based on Laboratory Parameter Variability When All Values for a Subject Were Below the Cutoff

Laboratory Parameter		Cutoff (xULN)	Subjects (n)	xBaseline (ULN), Using 95% CIs	
				Baseline = Mean	Baseline = Minimum
Chemistry	ALT	1.5	2571	1.4	1.8
Chemistry	C-peptide	1.5	821	1.5	2.0
Chemistry	Creatinine	1.0	2437	1.1	1.3
Chemistry	GGT	1.5	1259	1.4	1.7
Chemistry	Glucagon	1.0	663	1.4	1.9
Chemistry	Insulin	2.0	703	1.6	2.2
Chemistry	Lipase	1.5	1317	1.3	1.7
Chemistry	Proinsulin	2.0	474	1.7	2.4
Chemistry	Triglycerides	1.5	2334	1.4	1.8
Chemistry	Urate	1.2	2136	1.2	1.4
Hematology	hA1C/Hg	1.0	921	1.1	1.2
Urinalysis	Albumin	10.0	1264	2.1	3.2
Urinalysis	Urea	1.5	824	1.3	1.6

Table 9 Expected Fold Baseline, Based on Laboratory Parameter Variability When at Least One Value for a Subject Was Above the Cutoff

Laboratory Parameter		Cutoff (xULN)	Subjects (n)	xBaseline (ULN), Using 95% CIs	
				Baseline = Mean	Baseline = Minimum
Chemistry	ALT	1.5	254	1.8	2.6
Chemistry	C-peptide	1.5	83	2.0	3.0
Chemistry	Creatinine	1.0	388	1.3	1.5
Chemistry	GGT	1.5	188	1.7	2.4
Chemistry	Glucagon	1.0	105	1.7	2.3
Chemistry	Insulin	2.0	177	2.4	3.7
Chemistry	Lipase	1.5	257	2.1	3.2
Chemistry	Proinsulin	2.0	74	2.0	3.0
Chemistry	Triglycerides	1.5	461	1.7	2.4
Chemistry	Urate	1.2	167	1.3	1.6
Hematology	hA1C/Hg	1.0	1176	1.1	1.3
Urinalysis	Albumin	10.0	167	2.5	3.9
Urinalysis	Urea	1.5	97	1.4	1.7

within-subject variability of PSoC individuals with diabetes, one would expect to see up 2.6 x-baseline if the baseline value was based on one measure (potentially the minimum value for that subject) or up to 1.8 x-baseline if the baseline is based on multiple measures. Using either one of the cutoffs based on the PSoC data, the CTCAE grade 1 criteria will capture false-positive signals, values between the 1.5 x-baseline of the CTCAE definition and the 1.8 or 2.6 x-baseline based on the PSoC data.

Conclusion and Next Steps

These data clearly suggest that many laboratory parameters in individuals with diabetes were similar to the HVRR but some (~28%) were potentially different from the HVRR. The data also suggest that the 1) laboratory parameters potentially different from the HVRR that a x-baseline value could help identify the upper limit of expected values and 2) CTCAE v5.0 grade 1 criteria of 1.5–3.0 x-baseline if the baseline is abnormal may capture false-positive signals in clinical trials of individuals with diabetes. Further studies with higher subject numbers will be required to determine if the 28% of laboratory parameters are different from the HVRR and what criteria should be used for signal detection/identification. Limitations of this study that need to be addressed in future studies to determine the differences between

HVRR and patient populations and if the identification of laboratory parameter clinical safety signals can be improved include: 1) data from healthy volunteers to determine if the 10% cutoff used in this study was appropriate, 2) data from other patient populations to determine if the x-baseline values identified in individuals with diabetes are specific for this population or if the x-baseline is similar in all patient populations where the laboratory parameter is different from the HVRR, 3) determining if standard of care treatment and/or comorbidities explain increased lab values and should result in these individuals being evaluated differently than the other individuals in this population; 4) determining the impact of age, gender, and ethnicity; 5) determining which biomarkers are different from the HVRR based on statistical analysis and medical relevance; 6) evaluating a larger confirmatory dataset; 7) robust statistical analysis of x-baseline upper limit expected value; and 8) if within-subject variance is also due to disease progression.

Disclosure

All authors were employees of AstraZeneca Pharmaceuticals during the conduct of the study. The authors report no other conflicts of interest in this work.

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