STATISTICAL ANALYSIS PLAN

Study Title:	A Phase 2, Open-label, Translational Biology Study of Momelotinib in Transfusion-Dependent Subjects with Primary Myelofibrosis (PMF) or Post-polycythemia Vera or Post-essential Thrombocythemia Myelofibrosis (Post-PV/ET MF)
Name of Test Drug:	Momelotinib (MMB)
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LIST OF ABBREVIATIONS

AE	adverse event
ADME	absorption, distribution, metabolism and elimination
ALT	alanine aminotransferase
ANC	absolute neutrophil count
AST	aspartate aminotransferase
ATC	Anatomical-Therapeutic-Chemical (drug coding system)
BCRP	breast cancer resistant protein
BLQ	below the limit of quantitation
BMI	body mass index
BUN	blood urea nitrogen
CBC	complete blood count
CFR	Code of Federal Regulations
CI	confidence interval
CL_{cr}	creatinine clearance
CML	chronic myelogenous leukemia
CR	complete remission
CK	creatine kinase
CRF	case report form
CRO	contract (or clinical) research organization
CRP	C-reaction protein
CSR	clinical study report
CT	computed tomography/computed axial tomography scan
CTCAE	Common Terminology Criteria for Adverse Events
CYP450	cytochrome P450
Da	dalton
DIPSS	Dynamic International Prognostic Scoring System
DMC	data monitoring committee
DSPH	Drug Safety and Public Health
ECG	electrocardiogram
eCRF	electronic case report form(s)
EDC	electronic data capture
eDiary	electronic diary
EOT	end of treatment
EPO	erythropoietin
ESDD	Early Study Drug Discontinuation
ET	essential thrombocythemia
FAS	Full Analysis Set
FDA	(United States) Food and Drug Administration

FSH	follicle stimulating hormone
GCP	Good Clinical Practice(guidelines)
Gilead	Gilead Sciences
GLP	Good Laboratory Practice(guidelines)
hCG	human chorionic gonadotropin
Hgb	hemoglobin
HLGT	high-level group term
HLT	high-level term
HIV	human immunodeficiency virus
IB	investigator's brochure
ICH	International Conference on Harmonisation (of Technical Requirements for Registration of Pharmaceuticals for Human Use)
ID	subject identification
IP	investigational product
IPSS	International Prognostic Scoring System
ITT	intent to treat
IWG	International Working Group
LIC	liver iron concentration
LLOQ	lower limit of quantitation
MedDRA	Medical Dictionary for Regulatory Activities
MF	myelofibrosis
MMB	momelotinib
MPN	myeloproliferative neoplasm
MPN-SAF TSS	Myeloproliferative Neoplasm Symptom Assessment Form Total Symptom Score
MRI	magnetic resonance imaging
MST	MedDRA Search Term
ORR	overall response rate
PGIC	Patient Global Impression of Change
PK	pharmacokinetic
PMF	primary myelofibrosis
PP	per protocol
PRO	patient-reported outcome
PT	preferred term
PV	polycythemia vera
Q1, Q3	first quartile, third quartile
RBC	red blood cell
SAP	statistical analysis plan
SE	standard error
SI (units)	international system of units
SOC	system organ class

SRR24	splenic response rate at Week 24
StD	standard deviation
TD	transfusion-dependent/ dependence
TEAE	treatment-emergent adverse event
TFLs	tables, figures, and listings
TI	transfusion-independent/independence
TSAT	transferrin saturation
TSS	total symptom score
U	units
ULN	upper limit of normal
WHO	World Health Organization

1. INTRODUCTION

This statistical analysis plan (SAP) describes the statistical analysis methods and data presentations to be used in tables, figures, and listings (TFLs) in the clinical study report (CSR) for Study GS-US-352-1672. This SAP is based on the study protocol dated 18 May 2015 and the electronic case report form (eCRF). The SAP will be finalized before database finalization. Any changes made after the finalization of the SAP will be documented in the CSR.

1.1. Study Objectives

The primary objective of this study is as follows:

- To determine the transfusion independence response rate for transfusion dependent myelofibrosis (MF) subjects treated with momelotinib (MMB)

The secondary objectives of this study are as follows:

- To evaluate baseline levels and changes in markers of iron metabolism in transfusion dependent MF subjects treated with MMB
- To assess inhibition of JAK1/2 in transfusion dependent MF subjects treated with MMB
- To evaluate MMB pharmacokinetics (PK) in transfusion dependent MF subjects
- To evaluate changes in circulating cytokine and inflammatory markers in transfusion dependent MF subjects treated with MMB

1.2. Study Design

1.2.1. Design Configuration and Subject Population

This is a Phase 2, single-arm, open-label study of MMB to determine the transfusion independent response rate for transfusion-dependent subjects with primary myelofibrosis (PMF) or post-polycythemia vera or post-essential thrombocythemia myelofibrosis (Post-PV/MF). Study candidates will be adult males or females who are transfusion dependent with PMF or Post-PV/MF.

Eligible subjects will receive MMB tablet once daily for 24 weeks (± 7 days) on study. The starting dose of MMB for all subjects will be 200 mg tablet. The dose of MMB may be withheld or adjusted according to the criteria in the protocol.

Study visits will be completed at 2 weeks and 4 weeks following the initiation of MMB treatment, and every 4 weeks thereafter until Week 24. Following the screening, subjects will receive an electronic diary (eDiary) for daily completion of the modified Myeloproliferative Neoplasm Symptom Assessment Form Total Symptom Score (MPN-SAF TSS). Subjects will

complete the modified MPN-SAF TSS daily starting from screening and through 24 weeks of study participation. Laboratory analyses (serum chemistry, liver tests, hematology, urinalysis, pregnancy testing for females of childbearing potential), vital signs, adverse events (AEs), concomitant medications and disease assessments will be completed at regular study visits as defined in Appendix 3.

After completion of the 24-week (± 7 days) treatment phase visit procedures, subjects who respond to treatment, at the investigator's discretion, will have the option of continuing on maintenance therapy with MMB on Study GS-US-352-1154 at the MMB dose level they tolerated and/or derived clinical benefit from during the 24-week treatment period.

1.2.2. Number of Subjects Planned

Approximately 40

1.2.3. Inclusion Criteria

Subjects must meet all of the following inclusion criteria to be eligible for participation in this study:

1) Age ≥ 18 years old
2) Diagnosis of PMF or Post PV/ET-MF
3) Requires myelofibrosis therapy, in the opinion of the investigator
4) High risk or intermediate-2 risk defined by the Dynamic International Prognostic Scoring System (DIPSS) or intermediate-1 risk defined by DIPSS and associated with symptomatic splenomegaly and/or hepatomegaly
5) Transfusion dependent at baseline, defined as ≥ 4U red blood cell (RBC) transfusion in the 8 weeks prior to first dose of MMB
6) Acceptable organ function as evidenced by the following:
    a) Platelet Count $\geq 50 \times 10^9$/L
    b) AST/SGOT and ALT/SGPT ≤ 3 x upper limit of normal (ULN) or AST/SGOT or ALT/SGPT ≤ 5 x ULN if liver is involved by disease process as judged by the investigator
    c) Serum creatinine ≤ 2.0 mg/dL or calculated creatinine clearance of ≥ 60mL/min
    d) Direct bilirubin ≤ 2.0 x ULN
7) Peripheral blood blast count < 20%
8) Life expectancy of > 24 weeks

9) A negative serum pregnancy test for female subjects (unless surgically sterile or greater than two years postmenopausal)

10) Male subjects and female subjects of childbearing potential who engage in heterosexual intercourse must agree to use protocol specified method(s) of contraception as described in Appendix 3 in the protocol

11) Lactating females must agree to discontinue nursing before MMB administration

12) Able to understand and willing to sign the informed consent form

1.2.4. Exclusion Criteria

Subject who meet any of the following exclusion criteria are not to be enrolled in this study:

1) Prior splenectomy

2) Splenic irradiation within 3 months prior to the first dose of MMB

3) Prior treatment with MMB

4) Known positive status of human immunodeficiency virus (HIV)

5) Chronic active or acute viral hepatitis A, B, or C infection (testing required for hepatitis B and C), or hepatitis B or C carrier

6) Use of strong CYP3A4 inducer within 2 weeks prior to the first dose of MMB

7) Uncontrolled intercurrent illness including, but not limited to, active uncontrolled infection, uncontrolled cardiac arrhythmia, or psychiatric illness/social situation that would limit compliance with study requirements as judged by treating physician

8) History of a prior diagnosis of any malignancy other than PMF or Post PV/ET-MF except for the following: adequately treated local basal cell or squamous cell carcinoma of the skin, cervical carcinoma in situ, superficial bladder cancer, asymptomatic prostate cancer without known metastatic disease and with no requirement for therapy or requiring only hormonal therapy and with normal prostate specific antigen for >1 year prior to enrollment, or any other cancer that has been in complete remission without treatment for ≥ 5 years prior to enrollment.

9) Treatment with a JAK inhibitor within 21 days of the planned first dose of MMB

10) Documented myocardial infarction or unstable/uncontrolled cardiac disease (eg, unstable angina, congestive heart failure [New York Heart Association > Class III]) within 6 months of randomization

11) Presence of peripheral neuropathy ≥ CTCAE Grade 2

12) Unwilling or unable to undergo Magnetic Resonance Imaging (MRI) per requirements (Please see the Section 6.2.11.2 in the protocol)

13) Unwilling to consent to genomics sampling

14) Pregnant or breast-feeding

15) Known hypersensitivity to the study investigational medicinal products, the metabolites, or formulation excipients

1.2.5. Efficacy Endpoints

Primary Endpoint

The primary endpoint is transfusion independence response rate by Week 24, defined as becoming transfusion independent for ≥ 12 weeks at any time on study. A subject is considered as transfusion independent on study if no RBC transfusion occurs in any 12 weeks during the 24-week treatment period.

Secondary Endpoints

The secondary endpoints are:

- Transfusion response rate by Week 24, defined as becoming not transfusion dependent for ≥ 8 weeks at any time on study
- Baseline and change in markers of anemia (eg, hepcidin, ferritin)
- Change in pharmacodynamic biomarker pSTAT3
- Splenic response rate at Week 24, defined as ≥ 35% reduction in spleen volume from baseline as measured by MRI/CT
- Response rate in total symptom score (TSS) at Week 24, defined as achieving a ≥ 50% reduction from baseline in TSS as measured by the modified MPNSAF TSS diary
- Pharmacokinetics parameters (C_{max}, C_{last}, $C_{tau,}$ and AUC_{last}, if available) for MMB
- Change in circulating cytokine and inflammatory markers (eg, IL-6, IL-8, IFN-gamma, TNF-alpha)

Exploratory Endpoints

The exploratory endpoints are:

- Baseline and change in markers of orthogonal or parallel signaling pathways (eg, RAS/MAPK, PI3K/AKT)

- Baseline and change in gene expression profiles in whole blood and somatic mutations in whole blood, if applicable.
- Baseline and change in immune subsets in whole blood
- Change in exploratory pharmacodynamics markers of signaling mediated by JAK1 and/or JAK2 (eg, pSTAT1, pSTAT5, and pSTAT6)

Pharmacokinetics

Blood samples will be collected at the time points specified in the protocol.

1.2.6. Safety

The overall safety profile of MMB will be characterized by the type, frequency, severity, timing of onset, duration, and relationship to MMB of any adverse events (AEs) or abnormalities of laboratory tests; serious adverse events (SAEs); or AEs leading to discontinuation of MMB.

1.3. Sample Size and Power

This study will enroll approximately 40 subjects. Due to the exploratory nature, the study is not designed to detect a specific effect size. A sample size of 40 subjects is considered adequate for this exploratory study.

2. TYPE OF PLANNED ANALYSIS

2.1. Data Monitoring Committee

This study does not have a data monitoring committee (DMC).

2.2. Interim Analysis

No formal interim efficacy analysis is planned.

2.3. Final Analysis

After all subjects have completed the study, outstanding data queries have been resolved, and the database has been cleaned and finalized, the final analysis of the data will be performed.

2.4. Follow-up Analysis

After the final analysis, additional supplemental analyses of efficacy and safety may be performed to satisfy regulatory requirements and to perform long-term efficacy, safety, and overall survival follow-up.

3. GENERAL CONSIDERATIONS FOR DATA ANALYSES

Analysis results will be presented using descriptive statistics. For categorical variables, the number and percentage of subjects in each category will be presented; for continuous variables, the number of subjects [n], mean, standard deviation [StD] or standard error [SE], median, first quartile [Q1], third quartile [Q3], minimum, and maximum will be presented.

All statistical tests will be 2-sided and performed at the 10% significance level unless otherwise specified.

Data collected in the study will be presented in by-subject listings for all enrolled subjects, regardless of whether or not they received MMB. All by-subject listings will be presented by subject identification (ID) number in ascending order, unless otherwise specified.

3.1. Analysis Sets

Analysis sets define the subjects to be included in an analysis. Analysis sets and their definitions are provided in this section. For each analysis set, the number and percentage of subjects eligible for inclusion, as well as the number and percentage of subjects who were excluded and the reasons for their exclusion, will be provided. A listing of reasons for exclusion from analysis sets will be provided by subject.

3.1.1. Safety Analysis Set

The Safety Analysis Set includes all subjects who receive ≥ 1 dose of MMB. Since this study is a non-randomized study, the Safety Analysis Set will be used for subject's characteristics, efficacy and safety endpoints, and study treatment administration. This is the primary analysis set for safety analyses.

3.1.2. Biomarker Analysis Set

The Biomarker Analysis Set consists of all subjects in the Safety Analysis Set who have the necessary baseline and on-study measurements to provide interpretable results for the specific parameters of interest. The Biomarker Analysis Set will be used for biomarkers and the correlation analyses between biomarkers and efficacy clinical endpoints.

3.1.3. Pharmacokinetic Analysis Set

The Pharmacokinetic (PK) Analysis Set includes all subjects who have been administered at least 1 dose of investigational drug and have at least 1 non-missing postdose concentration value for the corresponding analysis in serum or plasma.

3.2. Strata and Covariates

This study does not use a stratified randomization schedule in enrolling subjects.

3.3. Examination of Subject Subsets

The primary efficacy endpoints will be examined using the following subsets:

- Age (< 65 years and ≥ 65 years)
- Gender (male and female)
- Race (white and all other races)
- DIPSS assessment (Intermediate-1/2 and high risk)
- Type of MF (PMF, Post-PV MF, and Post ET MF)
- JAK2V617F mutation status at baseline (positive and negative)
- Hemoglobin level at baseline (<8g/dL and ≥8g/dL)
- TSS at baseline (<median and ≥median)
- Spleen volume at baseline (<median and ≥median)
- Hepcidin at baseline morning time point (<median and ≥median)
- Liver iron content at baseline (<median and ≥median)
- Transferrin saturation at baseline (<50% and ≥50%)

Pharmacokinetics endpoints, AEs and lab abnormalities may be examined in the following subgroups:

- Age (< 65 years and ≥ 65 years)
- Gender (male and female)
- Race (white and all other races)

3.4. Multiple Comparisons

All endpoint tests will be done at the significance level of 0.1 unless otherwise specified. No multiplicity adjustment will be made for testing, because the endpoints are considered exploratory in nature.

3.5. Missing Data and Outliers

3.5.1. Missing Data

In general, missing data will not be imputed unless methods for handling missing data are specified.

For missing last dosing date of MMB, imputation rules are described in Section 4.2.1. The handling of missing or incomplete dates for disease diagnosis is described in Section 5.3.2.1, for AE onset is described in Section 7.1.5.2, and for prior and concomitant medications in Section 7.4.1.

3.5.2. Outliers

Outliers will be identified during the data management and data analysis process, but no sensitivity analyses will be conducted. All data will be included in the data analysis.

3.6. Data Handling Conventions and Transformations

By-subject listings will be presented for all subjects in the primary analysis set that these listings are supporting and sorted by subject ID number, visit date, and time (if applicable). Data collected on log forms, such as AEs, will be presented in chronological order within subject.

Age (in years) on the date of the first dose of MMB will be used for analyses and presentation in listings.

If a subject was not dosed with MMB at all, the date the informed consent was signed will be used instead of the first dose date of MMB. For some countries, only birth year is collected on the CRF. In those cases, "01 January" will be used for the unknown birth day and month for the purpose of age calculation, unless age is captured on the CRF.

3.6.1. Data Handling for Non-PK Laboratory Data

Non-PK data that are continuous in nature but are less than the lower limit of quantitation or above the upper limit of quantitation will be imputed as follows:

- A value that is one unit less than the limit of quantitation will be used for calculation of descriptive statistics if the datum is reported in the form of "< x" (where x is considered the limit of quantitation). For example, if the values are reported as < 50 and < 5.0, values of 49 and 4.9, respectively, will be used for calculation of summary statistics. An exception to this rule is any value reported as < 1. For values reported as < 1 or < 0.1, a value of 0.9 or 0.09, respectively, will be used for calculation of summary statistics.

- A value that is one unit above the limit of quantitation will be used for calculation of descriptive statistics if the datum is reported in the form of "> x" (where x is considered the limit of quantitation). Values with decimal points will follow the same logic as above.

- The limit of quantitation will be used for calculation of descriptive statistics if the datum is reported in the form of "≤ x" or "≥ x" (where x is considered the limit of quantitation).

If methods based on the assumption that the data are normally distributed are not adequate, analyses may be performed on transformed or nonparametric analysis methods may be used, as appropriate.

3.6.2. Data Handling for PK Data

Natural logarithm transformation will be used for plasma/blood concentrations. Plasma concentration values that are below the limit of quantitation (BLQ) will be presented as "BLQ" in the concentration data listing. Values that are BLQ will be treated as 0 at predose time points, and one-half the value of the lower limit of quantitation (LLOQ) at post-baseline time points, where LLOQ is corrected for the dilution factor (i.e., reported LLOQ/dilution factor) for determination of summary and order statistics.

The following conventions will be used for the presentation of summary and order statistics:

- If at least 1 subject has a concentration value of BLQ for the time point, the minimum value will be displayed as "BLQ."

- If more than 25% of the subjects have a concentration data value of BLQ for a given time point, the minimum and Q1 values will be displayed as "BLQ."

- If more than 50% of the subjects have a concentration data value of BLQ for a given time point, the minimum, Q1, and median values will be displayed as "BLQ."

- If more than 75% of the subjects have a concentration data value of BLQ for a given time point, the minimum, Q1, median, and Q3 values will be displayed as "BLQ."

- If all subjects have concentration data values of BLQ for a given time point, all order statistics (minimum, Q1, median, Q3, and maximum) will be displayed as "BLQ."

3.6.3. Data Handling for Efficacy Endpoints

If there is a significant degree of non-normality for a continuous endpoint, analysis may be performed on log-transformed data or using nonparametric methods, as appropriate.

For MRI spleen response, TSS response, and transfusion-independence (TI) response subjects will be considered as non-responder and as transfusion-dependent (TD) for TD response status in the primary analysis, if subjects early discontinued in the MMB treatment phase prior to the visit or with missing assessments at the corresponding visit.

3.7. Visit Windows

3.7.1. Definition of Study Day

Study Day 1 will be defined as the day of first dose of MMB administration in the study.

Study day will be calculated from the date of first dose of MMB administration and derived as follows:

- For post dose study days: Assessment Date – First Dosing Date + 1
- For days prior to the first dose: Assessment Date – First Dosing Date

The last participation date is defined as:

- If subject did not roll over to the GS-US-352-1154 study: the last date from the datasets including BM_MF2 (bone marrow biopsy),COVLAB (Covance lab), ECG_EG(Electrocardiogram), EPRO_ERT (patient-reported outcomes), QS_PGIC(patient-reported outcomes), EX (MMB administration), IWG (IWG-MRT/ELN response assessment), LVSPQL_MF (Length of palpable spleen size), VISDT (visit date), VSPERF (vital signs), MRI_PAREXEL (MRI/CT Spleen Volume).
- If subject was rolled over to the GS-US-352-1154 study: as the last date from previous datasets but prior to or on the Day 1 of GS-US-352-1154
- If subject discontinued MMB treatment due to death, the date of death will be the last participation date in the study.

Note AE, CM (con meds), PR_TFSN (transfusion), LAB (local lab), LABHGB, LABHEM, PK_EX_SERIAL and PK_SERIAL will not be included in the derivation.

3.7.2. Analysis Windows

Subject visits might not occur on protocol-specified days. Therefore, for the purpose of analysis, observations will be assigned to analysis windows.

In general, the baseline value will be the last nonmissing value on or prior to the first dose date of MMB. If multiple measurements occur on the same day, the last nonmissing value prior to the time of first dose of MMB will be considered as the baseline value. If these multiple measurements occur at the same time or the time is not available, the average of these measurements (for continuous data) or the worst among these measurements (for categorical data) will be considered the baseline value.

3.7.2.1. Analysis Windows for Efficacy Analyses

For efficacy analyses in MMB treatment, observations will be assigned to analysis windows for efficacy analyses of the endpoints MRI splenic response, TSS response rate, hepcidin, anemia related biomarkers, erythropoietin, MRI liver iron content, C-reactive protein, and pharmacodynamics biomarkers, as provided in Table 3-1 through Table 3-7.

The 8-weeks period for RBC transfusion dependence status at baseline is defined as the period from Day -55 to Day 1.

Nominal Day referenced in the below Table 3-1 through Table 3-7 are the Study Day calculated from the date of first dose of MMB.

Table 3-1. Analysis Windows for Efficacy Endpoints MRI Spleen Volume Response Assessment and MRI Liver Iron Content

Nominal Visit	Nominal Day	Lower Limit	Upper Limit
Baseline for MRI Spleen Volume/Liver Iron Content	1	-30	1
Week 24[a]	169	141	197

a Due to the challenge of scheduling the imaging scan, assessment after a subject rolls over to the GS-US-352-1154 study and satisfying the analysis window will:
- be used for the corresponding nominal visit, if no assessment prior to or on the date of the first dose of MMB in the GS-US-352-1154 study is available in the analysis window;
- not be used for the corresponding nominal visit, when there are assessments from the GS-US-352-1672 study, prior to or on the date of the first dose of MMB in the GS-US-352-1154 study, and satisfying the corresponding analysis window; instead assessments from GS-US-352-1672 will be used.

Table 3-2. Analysis Windows for Efficacy Analyses of TSS Response Rate at Week 24

Nominal Visit	Nominal Day	Lower Limit	Upper Limit
Baseline for TSS	1	-7	-1
Week 24[a]	169	28 days period with last day as Day 162.	28 days period with last day as Day 176.

a Last 28 consecutive days with ≥ 20 available daily TSS, with the last day of the 28 days period searched backward from the earliest date of "Upper limit" or the last participation date, through "Lower Limit", on days with non-missing Daily TSS.

Table 3-3. Analysis Windows for Hepcidin

Nominal Visit	Nominal Day	Lower Limit	Upper Limit
Baseline	-1	-30	-1
Enrollment	1	1	1
Week 2	15	8	21
Week 4	29	22	42
Week 8	57	43	70
Week 12	85	71	98
Week 16	113	99	126
Week 20	141	127	154
Week 24	169	155	182

Table 3-4. Analysis Windows for Anemia Related Biomarkers[a]

Nominal Visit	Nominal Day	Lower Limit	Upper Limit
Baseline	1	-30	1
Week 2	15	8	21
Week 4	29	22	42
Week 8	57	43	70
Week 12	85	71	98
Week 16	113	99	126
Week 20	141	127	154
Week 24	169	155	182

a Anemia related biomarkers include iron related biomarkers (iron, iron binding capacity, ferritin, non-transferrin bound iron, transferrin saturation, soluble transferrin receptor) and hematology biomarkers (hemoglobin, hematocrit, platelets, WBC, differential, reticulocyte count, % blasts, erythropoietin). For erythropoietin, observations will be assigned to different analysis windows from the rest of anemia related biomarkers.

Table 3-5. Analysis Windows for Erythropoietin

Nominal Visit	Nominal Day	Lower Limit	Upper Limit
Baseline	1	-30	1
Week 8	57	29	85
Week 20	141	113	169

Table 3-6. Analysis Windows for C-reactive Protein

Nominal Visit	Nominal Day	Lower Limit	Upper Limit
Baseline	1	-30	1
Week 2	15	8	43
Week 12	85	57	113
Week 24	169	141	197

Table 3-7. Analysis Windows for Pharmacodynamics Biomarker pSTAT3

Nominal Visit	Nominal Day	Lower Limit	Upper Limit
Enrollment	1	1	1
Week 4	29	8	57
Week 24	169	141	197

3.7.3. Selection of Data in the Event of Multiple Records in an Analysis Window

Depending on the statistical analysis method, single values may be required for each analysis window. For example, change from baseline by visit usually requires a single value, whereas a time-to-event analysis would not require 1 value per analysis window.

If multiple non-missing numeric observations exist in an analysis window, records will be chosen based on the following rules if a single value is needed:

- For baseline, the last available record on or prior to the date of the first dose of MMB will be selected. If there are multiple records with the same time or no time recorded on the same day, average (arithmetic or geometric mean, as appropriate) will be used for the baseline value.
- For post-baseline visits:
  - The record closest to the nominal day for that visit will be selected.
  - If there are 2 records that are equidistant from the nominal day, the later record will be selected.
  - If there is more than 1 record on the selected day, the average will be taken, unless otherwise specified.

If multiple valid non-missing categorical observations exist in a window, records will be selected as follows:

- For baseline, the last available record on or prior to the date of the first dose of MMB will be selected. If there are multiple records with the same time or no time recorded on the same day, the value with the lowest severity will be selected (eg, normal will be selected over abnormal for safety electrocardiogram [ECG] findings).

- For post-baseline visits, if there are multiple records with the same time or no time recorded on the same day, the most conservative value within the window will be selected (eg, abnormal will be selected over normal for safety ECG findings).

4. SUBJECT DISPOSITION

4.1. Subject Enrollment and Disposition

A summary of subject enrollment and disposition will be provided. This summary will present the number of subjects screened, the number of subjects rescreened, the number of subjects enrolled, and the number and percentage of subjects in each of the categories listed below. For the "Treated", the denominator for the percentage calculation will be the total number of subjects enrolled for each column. For all other categories, the denominator for the percentage calculation will be the total number of subjects in the Safety Analysis Set for each column.

- Screened
- Treated (Safety Analysis Set)
- Completed MMB
- Did not complete MMB with reason for premature discontinuation of MMB
- Completed the study
- Did not complete the study with reason for discontinuation of study
- Entered the roll over study of GS-US-352-1154
- Biomarker analysis.
- Pharmacokinetics analysis

In addition, the total number of subjects who were enrolled in the study and the number and percentage of subjects in each of the disposition categories listed above will be displayed in a flow chart.

The following by-subject listings will be provided by subject ID number in ascending order to support the above summary tables:

- Reasons for discontinuation of MMB or study
- Reasons for screen failure (will be provided by screening ID number in ascending order)
- Lot number and kit ID

4.2. Extent of Exposure

Extent of exposure to MMB will be examined by assessing the total duration of exposure to MMB and the level of adherence to the MMB specified in the protocol.

4.2.1. Duration of Exposure to MMB

Total duration of exposure to MMB will be defined as last dosing date minus first dosing date plus 1, regardless of any temporary interruptions in MMB administration, and will be expressed in weeks using up to 1 decimal place (eg, 4.5 weeks).

When the stopping date of the study drug is missing or partial, the following rule will be used to impute the stopping date:

- If subjects are continuing on study drug, use the earliest of the date of death or data cutoff date or date of rolling over to GS-US-352-1154 study for analysis to impute the last dosing date for the calculation of the duration of exposure to study drug.

- If the stopping date is partial, then stopping date will be imputed with the earliest of the last date of the month (if month and year are available) or the last month of the year (if only year is available), the date of one day prior to the first dose in the rollover study, or the early study drug discontinuation date or date of death.

The total duration of exposure to MMB will be summarized using descriptive statistics (number of subjects [n], mean, StD, median, Q1, Q3, minimum, and maximum) and using the number (ie, cumulative counts) and percentage of subjects exposed through the following time periods:

Baseline (Day 1), Week 4 (Day 29), Week 8 (Day 57), Week 12 (Day 85), Week 16 (Day 113), Week 20 (Day 141), and Week 24 (Day 169).

Summaries will be provided for the Safety Analysis Set.

The number of subjects who have dose reduction or interruptions, and the reasons, will be summarized.

No inferential statistics will be provided.

4.2.2. Adherence to MMB

The total number of tablets administered will be summarized using descriptive statistics (n, mean, StD, median, Q1, Q3, minimum, and maximum).

The presumed total number of tablets administered to a subject will be determined by the data collected on the drug accountability CRF using the following formula:

$$\text{Total Number of Tablets Administered} = \left(\sum \text{No. of Tablets Dispensed}\right) - \left(\sum \text{No. of Tablets Not Administered}\right)$$

where the number of tablets not administered is the sum of (a) the number of doses returned and (b) the number of (reported) missed tablets that were not returned.

If returned tablets were missing for the last dispensed bottle at the data cutoff date for subjects who were continuing on study drug, it will be assumed that these subjects had received the correct number of or tablets at the time of the data cutoff date.

The average daily dose (mg) will be calculated using the following formula:

$$\text{Average Daily Dose (mg)} = \frac{\sum(\text{Daily Dose in mg})}{\text{Total Number of Days on Study Drug}}$$

Total Number of Days on Study Drug = Last Dosing Date – First Dosing + 1

The level of adherence with the MMB regimen while subjects were on treatment (i.e., on-treatment adherence) will be determined by the total amount of MMB administered relative to the total amount of MMB expected to be administered during subject's actual on-treatment period based on the MMB regimen.

The level of on-treatment adherence will be expressed in percentage using the following formula:

$$\text{On-Treatment Adherence}(\%) = \left(\frac{\text{Total Amount of MMB Administered}}{\text{MMB Expected to Be Administered on Treatment}}\right) \times 100$$

Study drug expected to be administered on treatment (mg) is the dose (mg) prescribed, taking into account for dose reduction and interruption (ie, if dose reduced or interrupted for certain period, the dose expected to be administered should be the reduced dose level or 0mg if interrupted for that particular period) until the date of study drug withdrawn prematurely or completion.

Descriptive statistics for the level of on-treatment adherence (n, mean, StD, median, Q1, Q3, minimum, and maximum) with the number and percentage of subjects belonging to adherence categories (eg, *< 75%, ≥ 75 to < 90%, ≥ 90%*) will be provided by treatment group for the Safety Analysis Set.

No inferential statistics will be provided.

A separate by-subject listing of MMB administration and MMB accountability will be provided by subject ID number (in ascending order) and visit (in chronological order).

4.3. Protocol Deviations

Subjects who did not meet the eligibility criteria for study entry will be summarized regardless of whether they were exempted by the sponsor or not. The summary will present the number and percentage of subjects who did not meet at least 1 entry criterion and the number of subjects who violated specific entry criteria based on all enrolled subjects. A by-subject listing will be provided for those subjects who violated at least 1 inclusion or exclusion criterion. The listing will present the entry criterion (or criteria if more than 1 violation) that subjects did not meet and related comments, if collected.

Protocol deviations occurring after subjects entered the study are documented during routine monitoring. The number and percentage of subjects with important protocol deviations by deviation reason (eg, nonadherence to MMB, violation of inclusion/exclusion criteria) will be summarized for the all enrolled subjects. A by-subject listing will be provided for those subjects with any protocol deviation.

5. BASELINE CHARACTERISTICS

5.1. Demographics

Subject demographic variables (ie, age, sex, race, and ethnicity) will be summarized using descriptive statistics (n, mean, StD, median, Q1, Q3, minimum, and maximum) for age, and using the numbers and percentages of subjects for gender, age (<65 yrs vs ≥65 yrs), race, and ethnicity. Age is calculated in years at the date of initial MMB administration. If a subject did not receive MMB after enrollment, the subject's age will be calculated from the date that the subject signed the informed consent form. The summary of demographic data will be provided for the Safety Analysis Set. Partial date of birth will be imputed per Section 5.3.2.1.

A by-subject demographic listing, which includes the date the informed consent was signed, will be provided by subject ID number in ascending order.

5.2. Other Baseline Characteristics

Other baseline characteristics include body weight (in kg), height (in cm), body mass index (BMI; in kg/m^2), and disease and baseline characteristics will include type of MF (PMF, Post-PV MF, or post-ET MF), spleen volume(cm^3), TSS, RBC units transfused within 8 weeks prior to enrollment, bone marrow fibrosis grade, cytogenetics findings, DIPSS risk level, hemoglobin level (continuous and categorical by <8 or ≥8 g/dL), WBC (x10^3/uL), ANC (x10^3/uL), Platelet count (x10^3/uL), JAK2V617F mutation status (positive or negative) at baseline. These baseline characteristics will be summarized using descriptive statistics (n, mean, StD, median, Q1, Q3, minimum, and maximum) for continuous variables and using the numbers and percentages of subjects for categorical variables. The summary of baseline characteristics will be provided for the Safety Analysis Set.

A by-subject listing of other baseline characteristics will be provided by subject ID number in ascending order.

5.3. Medical History

5.3.1. General Medical History

General medical history (ie, conditions not specific to the disease being studied) data will be collected at screening and listed only. A by-subject listing of general medical history will be provided by subject ID number in ascending order.

5.3.2. Myelofibrosis Disease History

Myelofibrosis disease history will be collected at screening for disease-specific conditions.

Time since disease diagnosis (years) will be calculated by (date of first dosing date of MMB - date of MF diagnosis + 1) / 365.25. Time since disease diagnosis will be summarized using summary statistics for a continuous variable. No inferential statistics will be generated.

Myelofibrosis disease type (PMF, post-PV MF, and post-ET MF) will be summarized by using number and percentage of subjects.

A by-subject listing of disease-specific conditions will be provided by subject ID number in ascending order.

5.3.2.1. Incomplete Dates

All partial dates of diagnosis and last regimen will be identified, and the partial dates will be imputed as follows:

- If day and month are missing but year is available, then the imputed day and month will be 01 Jan.
- If day is missing but the month and year are available, then the imputed day will be the first day of the month.
- Partial date will not be imputed if the year is missing.

5.4. Myelofibrosis Disease-Specific Prior Therapy

Myelofibrosis disease-specific prior medication, defined as any medication for the purpose of treating MF disease with a start date that is prior to the enrollment, will be summarized by preferred term (PT) (collected through concomitant medication eCRF page) using the number and percentage of subjects on the medication. A subject reporting the same medication more than once will be counted only once when calculating the number and percentage of subjects who received that medication. The summary will be ordered by descending overall frequency of PT term. For drugs with the same frequency, sorting will be done alphabetically. If a partial start date is entered, any medication with the month and year (if day is missing) or year (if day and month are missing) prior to or the same as the enrollment will be included in the prior medication summary. Medication with day, month and year all missing for start date will also be taken as MF disease-specific prior medication. All MF disease-specific prior medication will be provided in a by-subject listing sorted by subject ID number and administration date in chronological order.

6. EFFICACY ANALYSES

6.1. Primary Efficacy Endpoint

6.1.1. Definition of the Primary Efficacy Endpoint

The primary efficacy endpoint of this study is transfusion independence (TI) response rate by Week 24 defined as becoming transfusion independent for ≥12 weeks at any time on study.

Twelve-week transfusion independent response by Week 24: ≥84 days transfusion independence response for subjects who are transfusion dependent at baseline, where transfusion dependent is defined as receiving at least 4 units of RBC transfusion in the 8 weeks prior to screening (or first dose of MMB). The subject will be considered as 12-week transfusion independent in post-baseline if at least 1 of the following 4 criteria is satisfied:

1) There is a time duration of at least 84 days between the first dose of MMB and the first RBC transfusion in post-baseline, where the duration will be calculated as follows:

   Duration between the first dose of MMB to the first RBC transfusion in post-baseline (days) = Date of first RBC transfusion in post-baseline – Date of first dose of MMB + 1

2) There is a time duration of at least 84 days between 2 consecutive RBC transfusions in post-baseline, where the duration will be calculated as follows:

   Duration between the 2 consecutive RBC transfusions in post-baseline (days) = Date of the second RBC transfusion in the consecutive RBC transfusions in post-baseline – Date of the first RBC transfusion in the successive RBC transfusions in post-baseline + 1

3) There is a time duration of at least 84 days between the last RBC transfusion in post-baseline and the last visit date, where the duration will be calculated as follows:

   Duration between the last RBC transfusion in post-baseline and the last available visit in post-baseline (days) = Date of the last available visit in post-baseline – Date of the last RBC transfusion in post-baseline + 1

4) No transfusion during the entire study in post-baseline and the length of the study is at least 84 days, where the duration will be determined as follows:

   Duration between the date of first dose of MMB and the last available visit in post-baseline (days) = Date of the last available visit in post-baseline – Date of the first dose of MMB + 1

Twelve-week transfusion independent response by Week 12: a subject is considered as 12-week transfusion indepdent by Week 12, if he did not have any transfusion in the first 84 days on study.

Any enrolled subject in the Safety Analysis Set who did not meet any of the criteria listed above will be considered a 12-week transfusion independent non-responder.

Calculation of 12-week transfusion independent response rate by Week 24 and Week 12:

$$\hat{p}_{12-TF} =$$

$$100 \times \frac{Number\ of\ patients\ with\ twelve-week\ transfusion\ independent\ response}{Total\ number\ of\ enrolled\ patients\ in\ Safety\ Analysis\ Set}$$

The denominator for calculation of the percentage of 12-week transfusion independent rate by Week 24 and Week 12 included all enrolled subjects in Safety Analysis Set.

6.1.2. Statistical Hypothesis for the Primary Efficacy Endpoint

There is no formal hypothesis testing in this study due to the exploratory nature of the primary end points.

6.1.3. Analysis of the Primary Efficacy Endpoint

The purpose of the primary analysis is to investigate the 12-week transfusion independence response rate by Week 24 with the treatment of MMB. In order to evaluate the proportion, $\hat{p}_{12-TF}$, a conventional 2-sided $100(1-\alpha)\%$ exact confidence limits based on Clopper-Pearson method {Clopper 1934} for binominal distribution will be calculated, where α is the confidence level. Unless otherwise indicated, it is equal to 0.10 in this study. Number and percent will be provided for subjects with or without Hgb < 8g/dL during 12-week transfusion-free window.

6.1.4. Exploratory Analysis

An exploratory analysis will be performed to investigate the potential prognostic factors influencing transfusion independent response rate using the logistic regression model approach. The variables with prognostic potential will be included in the model to identify plausible significant factors on transfusion independent response rate:

- Age (< 65 years and ≥ 65 years)
- Gender (male and female)
- Race (white and all other races)
- DIPSS assessment (Intermediate-1/2 and high risk)
- Type of MF (PMF, Post-PV MF and Post ET MF)
- JAK2V617F mutation status at baseline (positive and negative)
- Hemoglobin level at baseline (<8g/dL and ≥8g/dL)

- TSS at baseline (<median and ≥median)
- Spleen volume at baseline (<median and ≥median)
- Hepcidin at baseline morning time point (<median and ≥median)
- Liver iron content at baseline (<median and ≥median)
- Transferrin saturation at baseline (<50% and ≥50%)

Each prognostic factor will be preliminarily evaluated in the logistic regression model. Only the variables with significance level less than 0.2 will be considered to build the multivariate model. A stepwise selection process with significance level of 0.2 for entering variables and significance level of 0.10 for keeping variables will be applied to those candidate variables to identify the final subset of relevant covariates in the logistic regression model.

6.1.4.1. Duration of TI Response (DOR-TI)

Duration of TI response is defined as the interval from the first onset date of TI response to the earliest onset date of loss of TI response or censoring date among subjects who achieved TI response, ie, duration of TI response (weeks) = (date of loss of TI response or censoring – date of first TI response + 1) / 7. The loss of TI response is defined as the date of the first transfusion post the TI response. Subjects who remain TI will be censored at the last participation date.

Duration of TI response will be summarized using Kaplan-Meier method for TI response by Week 24 only. Number of subjects with events and subjects censored, median, Q1, Q3, min and max estimates will be presented.

6.1.4.2. Time to TI Response (TtTI)

Time to TI response (TtTI) among subjects who achieved TI response is defined as the interval from the date of the first dose of MMB administration to the first onset date of TI response, ie, time to TI (weeks) = (the date of first TI response onset – date of the first dose of MMB + 1) / 7. No censoring is needed.

Time to TI response will be summarized only for TI response by Week 24 through descriptive statistics (n, mean, StD, median, Q1, Q3, minimum, and maximum).

6.2. Secondary Efficacy Endpoints

6.2.1. Definition of Secondary Efficacy Endpoints

The following endpoints of this study are designated as secondary endpoints:

6.2.1.1. Transfusion Response Rate for ≥ 8 Weeks

Transfusion response for ≥ 8 weeks: defined as no RBC transfusions for at least 8 weeks at any time on study. A subject will be considered to achieve transfusion response for ≥ 8 weeks post-baseline if at least one of the following 2 criteria is satisfied:

(1) If there is no any transfusion event post-baseline and the length of study is ≥ 56 days.

Duration between dates in post-baseline = the last participant date – first dose date of MMB + 1.

(2) If there is any time interval between any 2 consecutive transfusion events post-baseline, or the time interval between the first dose of MMB date and the first transfusion date post-baseline, or the last transfusion date and the time interval between the last available visit date≥56 days

Duration between dates in post-baseline = later date – former date + 1.

6.2.1.2. Splenic Response Rate at Week 24

Splenic response rate at Week 24: defined proportion of subjects who achieve a ≥ 35% reduction in spleen volume at Week 24 (SRR24) from baseline as measured by MRI. The spleen volume will be obtained through the assessment of MRI scan by a central imaging laboratory described in the Section 6.2.11.2 in the protocol.

The baseline spleen volume is defined as the last spleen volume measured by MRI from the baseline period prior to or on the date of first dose of MMB administration (Day -30 to Day 1, Day 1 is defined as the day of first dose of MMB administration in the study).

The Week 24 spleen volume is defined as the spleen volume measurement by MRI at Week 24 (Day 141 to 197). When multiple records are existent between Day 141 and Day 197, the rules specified in Section 3.7.3 are applied. Due to the challenge of scheduling the imaging scan, assessment after the first MMB dose in the rollover study and between Day 141 to 197 for the subjects who were rolled over to Study GS-US-352-1154 will:

- Be used for Week 24 spleen volume, if no assessment prior to or on the date of the first rollover study MMB dosing and between Day 141 and Day 197 is available;
- Not be used for Week 24 spleen volume, if there are assessments in Study GS-US-352-1672, prior to or on the date of first rollover study MMB dosing and between Day 141 and Day 197; instead assessments in Study GS-US-352-1672 will be selected per rules specified in Section 3.7.3.

The %Change in spleen volume from baseline to Week 24 is calculated by:

$$\%change = 100 \times \frac{Week\ 24\ Spleen\ Volume - Baseline\ Spleen\ Volume}{Baseline\ Spleen\ Volume}$$

6.2.1.3. Response Rate of Total Symptom Score (TSS) at Week 24

The modified MPN-SAF TSS is an 8-item questionnaire developed to assess symptom burden and quality of life in patients with myeloproliferative neoplasms (MPNs). The TSS is assessed over time to evaluate changes in MPN-related symptoms. The modified MPN-SAF TSS is completed daily on an electronic diary (eDiary) device. NOTE: scores for the question of inactivity in the diary will not be included in TSS assessments.

The proportion of subjects who have achieved ≥ 50% reduction from baseline to Week 24 in TSS based on the modified MPN-SAF TSS will be determined as follows.

The diary is completed at the end of each day with a 24-hour recall period, and the daily TSS is defined as the sum of 7 individual symptom scores (each with a 0 to 10 point scale) collected on the same day. The daily TSS will be missing if there are any missing individual scores. If multiple records are available on the same day, the last record will be used as an assessment for that day.

The baseline TSS is defined as the average of the daily TSS from the 7-day baseline period (Day −7 through Day −1). The baseline TSS will be missing if there are more than 3 days of missing daily TSS during the 7-day baseline period.

The Week 24 TSS is defined as the average of the daily TSS from a consecutive 28-day period at Week 24. The consecutive 28-day period at Week 24 is defined as the latest eligible period of 28 consecutive days that has ≥ 20 available daily TSS; the last day of that 28-day period must be prior to or on the last participation date, have nonmissing daily TSS, and fall between Days 162 and 176, inclusive. If no such consecutive 28-day period with ≥ 20 available daily TSS is available or the last participation day was prior to Day 162, the Week 24 TSS will be considered missing.

The percentage change in TSS from baseline to Week 24 is calculated as follows:

%Change = 100 × (Week 24 TSS – Baseline TSS) / Baseline TSS

6.2.1.4. Change in Hepcidin

Hepcidin is measured twice at each study visit, one predose in the morning and one 6 hours postdose. Hepcidin is measure at the Baseline Visit (both morning and 6 hour samples are predose), at the Enrollment Visit and Weeks 2, 4, 8, 12, 16, and 24. Change and % change from baseline will calculated for predose and postdose values, respectively. Hepcidin value is obtained through the assessment by hepcidin analysis (Radbound).

The baseline visit for hepcidin is defined as the last visit in the baseline period prior to the date of first dose of MMB administration (Day -30 to Day -1, Day 1 is defined as the day of first dose of MMB administration in the study). Hepcidin value at predose time point in the morning at the baseline visit will be used as the baseline value for predose hepcidin measurement. Hepcidin value at 6 hours postdose at the baseline visit will be used as the baseline value for 6 hours postdose hepcidin measurement.

The post-baseline visits for hepcidin are defined using the analysis window specified in Section 3.7.2. Predose value and 6 hours postdose value at each post-baseline visit will be used to calculate the change and % change from baseline for hepcidin measured at predose time point and 6 hours postdose.

Daily hepcidin change will also be calculated at each study visit using the formula below:

$$Daily\ hepcidin\ change = 6\ hours\ postdose\ hepcidin\ value - predose\ hepcidin\ value$$

Daily hepcidin change at the baseline visit will be used as the baseline value for daily hepcidin change. Daily hepcidin change at post-baseline visits is defined by the analysis window specified in Section 3.7.2. Change and % change from baseline of daily hepcidin change will be calculated.

6.2.1.5. Baseline and Change in Anemia Related Biomarkers

Change and % change from baseline will be calculated for anemia related biomarkers, including iron related biomarkers (iron, iron binding capacity, ferritin, non-transferrin bound iron, transferrin saturation, and soluble transferrin receptor) and hematology biomarkers (hemoglobin, hematocrit, platelets, WBC, RBC, reticulocyte count, reticulocyte/erythrocyte % , % blasts, and erythropoietin). The values of these biomarkers are obtained from the central laboratory assessment as described in Protocol Section 6.2.7.

The baseline biomarker value is defined as the last value from the baseline period prior to or on the date of first dose of MMB administration (Day -30 to Day 1, Day 1 is defined as the day of first dose of MMB administration in the study).

The post-baseline biomarker value is defined as the value measured within the analysis windows of post-baseline visits specified in Section 3.7.2. When multiple records are in the analysis window of a post-baseline visit, the rules specified in Section 3.7.3 are applied.

6.2.1.5.1. Anemia Related Biomarker for TI Responders

For TI responders, change and % change from pre-TI response will be calculated for anemia related biomarker values during the period of TI response. Anemia related biomarker for this analysis include predose hepcidin, hepcidin 6 hour postdose, hepcidin daily change, iron, iron binding capacity, ferritin, non-transferrin bound iron, transferrin saturation, soluble transferrin receptor, hemoglobin, hematocrit, platelets, WBC, RBC, reticulocyte count, reticulocyte/erythrocyte %, % blasts, and erythropoietin.

The pre-TI response anemia biomarker value is defined as the last value prior to the start of the TI response window defined in Section 6.1.1. Anemia related biomarker values used for pre-TI response and during the period of TI response should be chosen from the baseline and post-baseline anemia related biomarker values defined above, and change and % change from pre-TI response will be calculated at 2 weeks, 4 weeks, 8 weeks, 12 weeks etc., after the pre-TI response anemia related biomarker value.

6.2.1.6. Change in Liver Iron Content

Change and % change from baseline will be calculated for liver iron content. The liver iron content is obtained from the central imaging laboratory MRI assessment described in Protocol Section 6.2.8.2.

The baseline liver iron content is defined as the last value from the baseline period prior to or on the date of first dose of MMB administration (Day -30 to Day 1, Day 1 is defined as the day of first dose of MMB administration in the study).

The Week 24 liver iron content is defined as the value measured within the analysis window of the Week 24 visit specified in Section 3.7.2. When multiple records are in the analysis window of a post-baseline visit, the rules specified in Section 3.7.3 are applied.

6.2.1.7. Change in C-reactive Protein

Change and % change from baseline will be calculated for C-reactive protein. The value of C-reactive protein is obtained from the central laboratory assessment described in Protocol Section 6.2.7.

The baseline C-reactive protein value is defined as the last value from the baseline period prior to or on the date of first dose of MMB administration (Day -30 to Day 1, Day 1 is defined as the day of first dose of MMB administration in the study).

The post-baseline C-reactive protein value is defined as the value measured within the analysis window of post-baseline visits specified in Section 3.7.2. When multiple records are in the analysis window of a post-baseline visit, the rules specified in Section 3.7.3 are applied.

6.2.1.8. Change in Pharmacodynamics Biomarker pSTAT3

Change and % change from baseline will be calculated for the pharmacodynamics biomarker pSTAT3, including %pSTAT Stimulated CD3+/4+ T cell and ratio between %pSTAT Stimulated CD3+/4+ T cell and %tSTAT Stimulated CD3+/4+ T cell. Ratio between %pSTAT Stimulated CD3+/4+ T cell and %tSTAT Stimulated CD3+/4+ T cell is calculated as 100%× (%pSTAT Stimulated CD3+/4+ T cell ÷ %tSTAT Stimulated CD3+/4+ T cell). pSTAT3 is measured at time points of predose, 2, 4 and 6 hour postdose at Enrollment, Week 4 and 24. The value of pSTAT3 is obtained from the central laboratory assessment described in Protocol Section 6.2.

The baseline pSTAT3 value is defined as the last predose value from the baseline period prior to or on the date of first dose of MMB administration (Day -30 to Day 1, Day 1 is defined as the day of first dose of MMB administration in the study).

The Week 4 and 24 pSTAT3 values are defined as values measured within the analysis windows of the Week 4 and 24 visits specified in Section 3.7.2. When multiple records are in the analysis window of a post-baseline visit, the rules specified in Section 3.7.3 are applied.

6.2.2. Analysis Methods for Secondary Efficacy Endpoints

Similar with the analysis method for the primary efficacy endpoint, the primary analyses of all of the secondary efficacy endpoints will be conducted using the Safety Analysis Set.

6.2.2.1. Transfusion Response Rate ≥ 8 weeks

Calculation of the transfusion response rate by Week 24:

$$\hat{p}_{8-TF} = 100 \times \frac{Number\ of\ Responders}{Total\ number\ of\ enrolled\ subjects\ in\ the\ Safety\ Analysis\ Set}$$

The denominator for calculation of the transfusion response rate by Week 24 includes all enrolled subjects in Safety Analysis Set.

The proportion, $\hat{p}_{8-TF}$, with a conventional 2-sided $100(1-\alpha)\%$ exact confidence limits based on Clopper-Pearson method for binominal distribution will be calculated.

6.2.2.2. Splenic Response Rate at Week 24

Calculation of the splenic response rate at Week 24:

$$\hat{p}_{spleen} =$$

$$100 \times \frac{\text{Number of subjects with} \geq 35\% \text{ spleen volume reduction at week 24}}{\text{Total number of enrolled subjects in the Safety Analysis Set}}$$

The denominator for calculation of the splenic response rate at Week 24 includes all enrolled subjects in Safety Analysis Set.

If spleen volume of a subject was not assessed by MRI scan between Study Day -30 and Day 1 (including Day 30 and Day 1), the subject will be coded as a non-responder of splenic response. Subjects who discontinue from the treatment before Week 24 and miss the Week 24 spleen volume measurement will also be considered as a non-responder.

The proportion, $\hat{p}_{spleen}$, with a conventional 2-sided $100(1-\alpha)\%$ exact confidence limits based on Clopper-Pearson method for binominal distribution will be calculated.

6.2.2.3. Response Rate in TSS

Calculation of the TSS response rate at Week 24:

$$\hat{p}_{TSS} = 100 \times \frac{\text{Number of subjects with} \geq 50\% \text{ TSS reduction at week 24}}{\text{Total number of enrolled subjects with non-missing baseline TSS in the Safety Analysis Set}}$$

Response rate in TSS at Week 24 will be analyzed for subjects who have a baseline TSS > 0 or subjects who have a baseline TSS = 0 but nonzero or missing TSS at Week 24. Subjects with missing Week 24 TSS will be considered as non-responders.

The proportion, $\hat{p}_{TSS}$, with a conventional 2-sided $100(1-\alpha)\%$ exact confidence limits based on Clopper-Pearson method for binominal distribution will be calculated.

6.2.2.4. Change in Hepcidin

Descriptive summary statistics will be provided for actual value, change, and % change from baseline for hepcidin measured at predose and 6 hours postdose of each study visit by TI response. Exploratory plots, such as median-IQR plot of hepcidin value by visit and TI response, may be provided.

A linear mixed effects model may be fitted to change from baseline for hepcidin with visit, time point, and TI response as the fixed effects and subject as the random effects. Least square mean estimate and 2-sided $100(1-\alpha)\%$ confidence intervals (CI) will be calculated for change from baseline of hepcidin at the predose and 6 hour postdose time point at each post-baseline study visit. If necessary, log transformation may be applied to hepcidin value.

Descriptive summary statistics will be provided for daily hepcidin change, change, and % change from baseline for daily hepcidin change by study visit and TI response. Exploratory plots, such as median-IQR plot of % change from baseline of daily hepcidin change by visit and TI response, may be provided.

A linear mixed effect model may be fitted to change from baseline for daily hepcidin change with visit and TI response as the fixed effects and subject as the random effects. Least square means and 2-sided $100(1-\alpha)\%$ confidence intervals (CI) will be calculated for change from baseline of daily hepcidin change at each post-baseline study visit. If necessary, log transformation may be applied to daily hepcidin change.

6.2.2.5. Baseline and Change in Anemia Related Biomarkers

Descriptive summary statistics will be provided for actual value, change, and % change from baseline of anemia related biomarkers by visit and TI response. Exploratory plots, such as median-IQR plot of % change from baseline of anemia related biomarkers by visit and TI response, may be provided.

6.2.2.6. Change in Liver Iron Content

Descriptive summary statistics will be provided for actual value, change, and % change from baseline of liver iron content by visit and TI response.

6.2.2.7. Change in C-reactive Protein

Descriptive summary statistics will be provided for actual value, change, and % change from baseline of C-reactive protein by visit and TI response. Exploratory plots, such as median-IQR plot of % change from baseline of C-reactive protein by visit and TI response, may be provided.

6.2.2.8. Change in Pharmacodynamics Biomarker pSTAT3

Descriptive summary statistics will be provided for actual value, change, and % change from baseline of pSTAT3 at each time point and visit. Exploratory plots, such as median -IQR plot of % change from baseline of pSTAT3 by visit may be provided.

6.3. Exploratory Efficacy Endpoint

6.3.1. Definition of Exploratory Efficacy Endpoints

6.3.1.1. Duration of TI Response (DOR-TI)

Duration of TI response is defined as the interval from the first onset date of TI response to the earliest onset date of loss of TI response or censoring date among subjects who achieved TI response, ie, duration of TI response (weeks) = (date of loss of TI response or censoring – date of first TI response + 1) / 7. The loss of TI response is defined as the date of the first transfusion post the TI response. Subjects who remain TI will be censored at the last participation date.

Duration of TI response will be summarized using Kaplan-Meier method. Number of subjects with events and subjects censored, median, Q1, Q3, and min and max estimates will be presented.

6.3.1.2. Time to TI Response (TtTI)

Time to TI response (TtTI) among subjects who achieved TI response is defined as the interval from the date of the first dose of MMB administration to the first onset date of TI response, ie, time to TI (weeks) = (the date of first TI response onset – date of the first dose of MMB + 1) / 7. No censoring is needed

Time to TI response will be summarized through descriptive statistics (n, mean, StD, median, Q1, Q3, minimum, and maximum).

6.3.1.3. Leukemia-Free Survival

Leukemia-free survival (LFS) is defined as the interval first dose date to the date of first documented leukemic transformation or death from any cause, ie, LFS (weeks) = (date of censoring or date of earliest of leukemic transformation or death – first dose date + 1) / 7. Leukemic transformation was documented in long-term follow up and AE eCRF page. Leukemic transformation related AE PTs are listed in Appendix 6.

6.3.1.4. Bone Marrow Fibrosis Score

Bone marrow fibrosis improvement is reduction in bone marrow fibrosis score by one or more than one grade.

6.3.2. Analysis Methods for Exploratory Efficacy Endpoints

Time to event endpoints will be summarized using Kaplan-Meier method, with exception that TtTI will be summarized through descriptive statistics (n, mean, StD, median, Q1, Q3, minimum, and maximum). Number and percentage of subjects with events and censored subjects, medians and ranges of survival time will be presented. A plot of the Kaplan-Meier curves will be provided. Censoring for each time to event endpoints was specified in Section 3.6.3.

For bone marrow fibrosis score, number and percentage of subjects in each grade will be provided. Number and percentage of subjects whose bone marrow fibrosis improved to grade 0 from baseline bone marrow fibrosis greater than grade 0 will also be provided. Improving rate defined as proportion of subjects with bone marrow fibrosis improvement among subjects with non-missing baseline bone marrow fibrosis score will be calculated, a conventional 2-sided $100(1-\alpha)\%$ exact confidence limits based on Clopper-Pearson method for binominal distribution will be calculated, where α is the confidence level.

Corresponding listings will be provided.

6.4. Changes From Protocol-Specified Efficacy Analyses

Pharmacokinetics parameters (C_{max}, C_{last}, C_{tau} and AUC_{last}, if available) for MMB mentioned in Protocol Section 8.1.3 have been removed as an analysis endpoint in the SAP due to the sparse PK samples collection.

Change in circulating cytokine and inflammatory markers (eg, IL-6, IL-8, IFN-gamma, TNF-alpha) have been removed as an analysis endpoint in the SAP.

The following exploratory endpoints have been removed as analysis endpoints in the SAP

- Baseline and change in markers of orthogonal or parallel signaling pathways (eg, RAS/MAPK, PI3K/AKT)
- Baseline and change in gene expression profiles in whole blood and somatic mutations in whole blood, if applicable.
- Baseline and change in immune subsets in whole blood
- Change in exploratory pharmacodynamics markers of signaling mediated by JAK1 and/or JAK2 (eg, pSTAT1, pSTAT5, and pSTAT6)

7. SAFETY ANALYSES

7.1. Adverse Events and Deaths

7.1.1. Adverse Event Dictionary

Clinical and laboratory adverse events (AEs) will be coded using the current version of MedDRA. System organ class (SOC), preferred term (PT) will be provided in the AE dataset.

7.1.2. Adverse Event Severity

Adverse events are graded by the investigator as Grade 1 (mild), 2 (moderate), 3 (severe), 4 (life-threatening), or 5 (fatal) according to the Common Terminology Criteria for Adverse Events [CTCAE] Version 4.03. The severity grade of events for which the investigator did not record severity will be categorized as "missing" for tabular summaries and data listings. The missing category will be listed last in summary presentation.

7.1.3. Relationship of Adverse Events to MMB

Related AEs are those for which the investigator selected "Related" on the AE case report form (CRF) to the question of "Related to Study Treatment." Relatedness will always default to the investigator's choice, not that of the medical monitor. Events for which the investigator did not record relationships to MMB will be considered related to MMB for summary purposes. However, by-subject data listings will show the relationship as missing from that captured on the CRF.

7.1.4. Serious Adverse Events

Serious adverse events (SAEs) will be identified and captured as SAEs if AEs met the definition of an SAE specified in the study protocol. Serious adverse events captured and stored in the clinical database will be reconciled with the SAE database from the Gilead Drug Safety and Public Health Department before database finalization.

7.1.5. Treatment-Emergent Adverse Events

7.1.5.1. Definition of Treatment Emergent

Treatment-emergent adverse events (TEAEs) are defined as 1 or both of the following:

- Any AEs with an onset date on or after the MMB start date and no later than 30 days after permanent discontinuation of MMB.
- Any AEs leading to premature discontinuation of MMB.

7.1.5.2. Incomplete Dates

If the onset date of the AE is incomplete and AE stop date is not prior to the first dosing date of MMB, the month and year (or year alone if month is missing too) of onset determine whether an AE is treatment emergent. The AE is considered treatment emergent if both of the following 2 criteria are met:

- The AE onset is the same as or after the month and year (or year) of the first dosing date of MMB, and
- The AE onset date is the same as or before the month and year (or year) of the date corresponding to 30 days after the date of the last dose of MMB.

An AE with completely missing onset and stop dates, or with the onset date missing and a stop date later than the first dosing date of MMB, will be considered to be treatment emergent.

7.1.6. Summaries of Adverse Events and Deaths

A brief high-level summary of TEAEs will be provided by the number and percentage of subjects who had the following: any AE; any AE of Grade 3 or above; any treatment-related AE; any treatment-related AE of Grade 3 or above; any SAE; any treatment-related SAE; any AE that led to premature discontinuation of MMB; and any AE that led to premature discontinuation of study; and any AE of interest. All deaths (including those that are treatment emergent and those that are not treatment emergent) observed will also be summarized and included in this table.

Adverse event summaries will provide the number and percentage of subjects with TEAEs by SOC, PT and Severity, using the Safety Analysis Set as follows:

- All AEs (by SOC, PT and Severity)
- All AEs of Grade 3 or above
- All treatment-related AEs
- All treatment-related AEs of Grade 3 or above
- All SAEs
- All treatment-related SAEs
- All AEs leading to premature discontinuation of MMB
- All AEs leading to a temporary interruption of MMB
- All AEs leading to a dose reduction of MMB

- All AEs leading to death (i.e., outcome of death)
- All AEs of interest
  - Peripheral Neuropathy SMQ (narrow terms only)
  - Cataract

First Dose Effect AE (defined as AE with PT of Dizziness, Flushing, Hot Flush, Headache, Hypotension, or Nausea, which occurred on the day of the first dosing date and resolved by the next day)In addition, all AEs recorded between screening and first dose of study drug will also be summarized for the Safety Analysis Set.

Multiple events will be counted only once per subject in each summary. The AEs will be summarized and listed first by SOC in descending order of incidence and then by PT, also in descending order of incidence, within each SOC of the investigational MMB group. For summaries by severity, the most severe grade will be used for those AEs that occurred in a given subject more than once during the study.

In addition to the presentation by SOC and PT, summaries will also be presented by PT only, ordered by decreasing total frequency for TEAEs that occurred in at least 5% of subjects within any treatment group, treatment-related TEAEs leading to discontinuation of IPs; TEAEs leading to dose modification or interruption of IP; treatment-emergent SAEs; and TEAEs of Grade >= 3 or above.

Summary of all TEAEs will also be presented by SOC, and PT for the following subgroups: gender (Male or Female), age group (<65 or >=65 years) and race (White or Non-White).

In addition to the summaries described above, data listings will be provided for the following:

- All AEs (with a variable indicating whether the event is treatment emergent)
- All AEs of Grade 3 or above
- SAEs
- Deaths
- All SAEs leading to death
- AEs leading to premature discontinuation of study drug
- AEs leading to premature discontinuation of study
- AEs leading to Dose interruption or modification of study drug

- AE related to study drug
- AEs of interest
  - Peripheral Neuropathy SMQ (narrow terms only)
  - Cataract
  - First Dose Effect

7.1.7. Additional Analysis of Adverse Events

7.1.7.1. Exposure-Adjusted Treatment-Emergent Adverse Events Rate

The exposure-adjusted TEAE rate is defined as the number of subjects with a specific event divided by the total exposure-time among the subjects in the treatment group and at risk of an initial occurrence of the event. Specifically,

$$Exposure - Adjusted\ TEAE\ Rate = \frac{n}{\sum t_i}$$

where n is the number of subjects with events; t_i is the interval (years) from first dosing date of study drug to the onset of first event for subjects with events, or will be censored at the time of the last participation date, or death date, or data cut-off date, whichever is earliest for subjects without events.

The exposure-adjusted TEAE rate will be summarized for all TEAEs, SAEs, ≥ Grade 3 AEs.

7.1.7.2. Time to Onset of AE of Interest (Peripheral Neuropathy TEAEs)

Time to onset of AE of interest is defined as the interval from the first dose of study drug to the onset date of the first event of the AE of interest, ie, calculated as (the onset date of the first event of the AE of interest or censoring date – date of the first dose of study drug + 1). In the absence of the occurrence of the AE of interest, the censoring date is the last participation date, or death date, or data cut-off date, whichever is earliest for subjects without events.

Summary and Kaplan-Meier plot will be provided for peripheral neuropathy TEAEs (narrow term only).

7.1.7.3. Time to Resolution of AE of Interest (Peripheral Neuropathy TEAEs)

Time to resolution of AE of interest is defined as the interval from the onset date of the first event of the AE of interest to the time that the last event of the AE of interest is resolved, ie, calculated as (the resolution date of the last event of the AE of interest or censoring date – the onset date of the first event of the AE of interest + 1). In the absence of the resolution of the AE of interest, the censoring date is the last participation date, or death date, or data cut-off date, whichever is earliest.

Summary and Kaplan-Meier plot will be provided for peripheral neuropathy TEAEs (narrow term only).

7.2. Laboratory Evaluations

Laboratory data collected during the study will be analyzed and summarized using both quantitative and qualitative methods. Summaries of laboratory data will be provided for the Safety Analysis Set. The analysis will be based on values reported in conventional units. When values are below the limit of quantitation, they will be listed as such, and the closest imputed value will be used for the purpose of calculating summary statistics. Hemolyzed test results will not be included in the analysis, but they will be listed in by-subject laboratory listings.

A by-subject listing for laboratory test results will be provided by subject ID number and visit in chronological order for hematology, serum chemistry, urinalysis and thiamine separately. Values falling outside of the relevant reference range and/or having a severity grade of 1 or higher on the CTCAE severity grade will be flagged in the data listings, as appropriate.

No inferential statistics will be generated.

7.2.1. Graded Laboratory Values

CTCAE Version 4.03 will be used for assigning toxicity grades (0 to 4) to laboratory results for analysis except for anemia (hemoglobin, Hgb) which will be graded using the CTCAE Version 3.0 as defined in Table 7-1. Grade 0 includes all values that do not meet the criteria for an abnormality of at least Grade 1. Some laboratory tests have criteria for both increased and decreased levels; analyses for each direction (i.e., increased, decreased) will be presented separately.

Table 7-1. Grading of Hemoglobin Severity

Grade (CTCAE 3.0)				
1	**2**	**3**	**4**	**5**
<LLN – 10.0 g/dL <LLN – 6.2 mmol/L <LLN – 100 g/L	<10.0 – 8.0 g/dL <6.2 – 4.9 mmol/L <100 – 80 g/L	<8.0 – 6.5 g/dL <4.9 – 4.0 mmol/L <80 – 65 g/L	<6.5 g/dL <4.0 mmol/L <65 g/L	Death

7.2.1.1. Treatment-Emergent (TE) Laboratory Abnormalities

Treatment-emergent laboratory abnormalities are defined as values that increase at least 1 toxicity grade from baseline at any post-baseline time point, up to and including the date of last dose of MMB plus 30 days for subjects who permanently discontinued MMB. If the relevant baseline laboratory value is missing, any abnormality of at least Grade 1 observed within the time frame specified above will be considered treatment emergent.

7.2.1.2. Treatment-Emergent Marked Laboratory Abnormalities

Treatment-emergent marked laboratory abnormalities are defined as values that increase from baseline by at least 3 toxicity grades at any post-baseline time point, up to and including the date of the last dose of MMB plus 30 days for subjects who permanently discontinued MMB. If the relevant baseline laboratory value is missing, any Grade 3 or 4 values observed within the timeframe specified above will be considered treatment-emergent marked abnormalities.

7.2.1.3. Summaries of Laboratory Abnormalities

The following summaries (number and percentage of subjects) for treatment-emergent laboratory abnormalities will be provided by lab test; subjects will be categorized according to the most severe post-baseline abnormality grade for a given lab test:

- All laboratory abnormalities
- Grade 3 or 4 laboratory abnormalities
- Marked laboratory abnormalities

For all summaries of laboratory abnormalities, the denominator is the number of subjects with nonmissing post-baseline values.

A by-subject listing of treatment-emergent Grade 3 or 4 laboratory abnormalities or marked laboratory abnormalities, if abnormalities were not graded will be provided by subject ID number and visit in chronological order. This listing will include all test results that were collected throughout the study for the lab test of interest, with all applicable severity grades or abnormal flags displayed.

7.2.2. Shifts Relative to the Baseline Value

Shift tables will be presented by showing change in CTCAE severity grade from baseline to the worst grade post-baseline grade. For lab tests for which a severity scale does not exist, shift tables will be presented showing change in results from baseline value (low, normal and high) to the worst post-baseline value (low, normal, high and “high & low”, where “high & low” is for lab tests with both high and low values at post-baseline visits).

7.3. Body Weight

Descriptive statistics (n, mean, StD, median, Q1, Q3, minimum, and maximum) will be provided for body weight as follows:

- Baseline value
- Values at each post-baseline time point
- Change from baseline at each post-baseline time point

- Post-baseline maximum value
- Change from baseline to post-baseline maximum value
- Post-baseline minimum value
- Change from baseline to post-baseline minimum value

A baseline value will be defined as the last available value collected on or prior to the date/time of first dose of MMB. Change from baseline to a post-baseline visit will be defined as the post-baseline value minus the baseline value. Body weight measured at unscheduled visits will be included for the baseline and post-baseline maximum and minimum value selection.

In the case of multiple values in an analysis window, data will be selected for analysis as described in Section 3.7.3. No inferential statistics will be generated.

Body weight will be summarized by grade, where grade is defined as follows:

The maximum grade of post-baseline weight gain and weight loss will be calculated and summarized based on the below table per CTCAE 4.03:

Post-baseline Maximum Grade of Weight Gain or Weight Loss	Grade	Criteria for Grade
Weight Gain	0	maximum % change from baseline < 5%
	1	5% ≤ maximum % change from baseline < 10%
	2	10% ≤ maximum % change from baseline < 20%
	3	maximum % change from baseline ≥ 20%
Weight Loss	0	-5% < minimum % change from baseline
	1	-10% < minimum % change from baseline ≤ -5%
	2	-20% < minimum % change from baseline ≤ -10%
	3	minimum % change from baseline ≤ -20%

7.4. Prior and Concomitant Medications

Medications collected at screening and during the study will be coded using the current version of the World Health Organization (WHO) Drug dictionary. The medications will be categorized as prior, concomitant, or both using the following definitions:

- Prior medications: any medications taken and stopped prior to or on the initial MMB dosing date based on the Prior and Concomitant Medication eCRF page
- Concomitant medications: any medications taken after the initial MMB dosing date and up to the last dosing date of MMB

7.4.1. Prior Medications and Concomitant Medications

Prior medications and concomitant medications will be summarized by Anatomical Therapeutic Chemical (ATC) drug class Level 2, Level 4 and preferred name using the number and percentage of subjects for each treatment group and overall. A subject reporting the same medication more than once will be counted only once when calculating the number and percentage of subjects who received that medication. The summary will be ordered by descending overall frequency of ATC drug classes and then by preferred names within an ATC drug class of the MMB treatment. For drugs with the same frequency, sorting will be done alphabetically.

Summaries will be based on the Safety Analysis Set. No inferential statistics will be generated.

For purposes of analysis, any medication with a stop date that is prior to or on the initial MMB dosing date will be included in the prior medication summary. If a partial stop date is entered, any medication with the month and year (if day is missing) or year (if day and month are missing) prior to the initial MMB dosing date will be included in the prior medication summary.

All prior and concomitant medications (other than per-protocol study drugs) will be provided in a by-subject listing sorted by subject ID number and administration date in chronological order.

7.5. Electrocardiogram Results

Electrocardiogram (ECG) data will not be presented in the CSR since ECGs were not assessed in this study other than as part of the screening process for potential new subjects.

7.6. Other Safety Measures

7.6.1. Overall Survival (OS)

OS, for subjects who died while on study, is defined as the interval from the date of the first dose of study drug to the date of the death from any cause, ie, the date of death– the first dose date +1.

Data from surviving subjects (ie. subjects who do not die while on study) will be censored at the last date that subject was known to be alive, ie, the last study participation date.

Overall survival will be summarized using the Kaplan-Meier method. A plot of the Kaplan-Meier curves will be provided. Number and percentage of subjects with events and censored subjects, medians and ranges of survival time will be presented.

A listing of overall survival will also be provided.

8. PHARMACOKINETIC ANALYSES

The concentration data of MMB and major metabolite GS-644603 , will be listed and summarized by nominal sampling time using descriptive statistics by study visit if applicable. Summary statistics (n, mean, StD, coefficient of variation [%CV], median, min, max, Q1, and Q3) will be presented.

Individual concentration data listings and summaries will include all subjects with concentration data. The sample size for each time point will be based on the number of subjects with nonmissing concentration data at that time point. The number of subjects with concentration BLQ will be presented for each time point.

The following tables will be provided for each analyte:

- Individual subject concentration data and summary statistics

The following figures may be provided for each analyte by visit:

- Mean (± StD) concentration data versus time (on linear and semilogarithmic scales)
- Median (Q1, Q3) concentration data versus time (on linear and semilogarithmic scales)
- Individual, mean, and median postdose concentration values that are ≤ LLOQ will not be displayed in the figures and remaining points connected.

The following listings will be provided:

- PK sampling details by subject including deviations in scheduled and actual draw times and procedures

9. REFERENCES

Clopper CJ, Pearson ES. The Use of Confidence or Fiducial Limits Illustrated in the Case of the Binomial. Dec. Biometrika 1934;26 (4):pp. 404-13.

10. SOFTWARE

SAS® Software Version 9.2. SAS Institute Inc., Cary, NC, USA.

nQuery Advisor(R) Version X.0. Statistical Solutions, Cork, Ireland.

11. SAP REVISION

Revision Date (DD MMM YYYY)	Section	Summary of Revision	Reason for Revision

12. APPENDICES

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16.2.7.1.2	Adverse Events for Peripheral Neuropathy SMQ	Safety Analysis Set	
16.2.7.1.3	Adverse Events for Cataract MST	Safety Analysis Set	
16.2.7.1.4	Adverse Events for First Dose Effect	Safety Analysis Set	
16.2.7.2.1	Deaths	All Screened Subjects	
16.2.7.2.2	Adverse Events Leading to Death	Safety Analysis Set	
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16.2.8.1.1.2	Hematology Results Part 2	Safety Analysis Set	
16.2.8.1.1.3	Hematology Results Part 3	Safety Analysis Set	
16.2.8.1.1.4	Hematology Results Part 4	Safety Analysis Set	
16.2.8.1.1.5	Hematology Results Part 5	Safety Analysis Set	
16.2.8.1.1.6	Hematology Results Part 6	Safety Analysis Set	
16.2.8.1.2.1	Serum Chemistry Results Part 1	Safety Analysis Set	
16.2.8.1.2.2	Serum Chemistry Results Part 2	Safety Analysis Set	
16.2.8.1.2.3	Serum Chemistry Results Part 3	Safety Analysis Set	
16.2.8.1.2.4	Serum Chemistry Results Part 4	Safety Analysis Set	
16.2.8.1.2.5	Serum Chemistry Results Part 5	Safety Analysis Set	
16.2.8.1.3.1	Urinalysis Results Part 1	Safety Analysis Set	
16.2.8.1.3.2	Urinalysis Results Part 2	Safety Analysis Set	
16.2.8.1.3.3	Urinalysis Results Part 3	Safety Analysis Set	
16.2.8.1.3.4	Urinalysis Results Part 4	Safety Analysis Set	

TFL Number	Title1	Title2	Title3
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16.2.8.1.5	Treatment-Emergent Marked Laboratory Abnormalities	Safety Analysis Set	
16.2.8.1.6	Treatment-Emergent Grade 3 or Above Laboratory Abnormalities	Safety Analysis Set	
16.2.8.2.1	Vital Signs	Safety Analysis Set	
16.2.8.2.2	Body Weight and Height	Safety Analysis Set	
16.2.8.3	Pregnancy Report	Safety Analysis Set	
16.3.1	Spleen Volume Measurement	Safety Analysis Set	
16.3.2.1	Response of TSS	Safety Analysis Set	
16.3.2.2	TSS Measurement	Safety Analysis Set	
16.3.2.3	Individual Score of MPN-SAF 2.0 by Every 4 Weeks	Safety Analysis Set	
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16.3.4.2.2	Anemia Biomarkers Part 2	Biomarker Analysis Set	
16.3.4.2.3	Anemia Biomarkers for TI Responder Part 1	Biomarker Analysis Set	
16.3.4.2.4	Anemia Biomarkers for TI Responder Part 2	Biomarker Analysis Set	
16.3.4.3	Liver iron content	Biomarker Analysis Set	
16.3.4.4	C-reactive Protein	Biomarker Analysis Set	
16.3.4.5	Phosphorylated STAT3	Biomarker Analysis Set	
16.3.4.6	Overall Survival	Safety Analysis Set	
16.3.4.7	Leukemia-Free Survival	Safety Analysis Set	
16.3.5	Patient Global Improvement of Change (PGIC)	Safety Analysis Set	

Appendix 2. Study Procedures Table

Assessment	Screening (-28 to -21 days)	Baseline (-7 +/- 2 days)	Enrollment	Week 2 (+/- 3 days)	Week 4 (+/- 3 days)	Week 8 (+/- 3 days)	Week 12 (+/- 3 days)	Week 16 (+/- 3 days)	Week 20 (+/- 3 days)	Week 24 (+/- 3 days)	Early Study Drug Discontinuation	30 Day Follow-Up
Informed consent	X											
Medication and medical history	X											
Transfusion recoding	X	X	X	X	X	X	X	X	X	X	X	X
Physical exam and myelofibrosis symptoms assessment	X	X	X	X	X	X	X	X	X	X	X	X
DIPSS assessment	X											
Vital signs	X	X	X		X	X	X	X	X	X	X	X
AEs, concomitant medications		X	X	X	X	X	X	X	X	X	X	X
Ophthalmic examination		X								X	X	
MMB accountability and dispensing[g]			X		X	X	X	X	X	X	X	
MMB Dosing at site			X	X	X	X	X	X	X	X		
Patient reporting outcomes												
Modified MPN-SAF TSS	←----------Daily----------→										X	
PGIC										X	X	

Assessment	Screening (-28 to -21 days)	Baseline (-7 +/- 2 days)	Enrollment	Week 2 (+/- 3 days)	Week 4 (+/- 3 days)	Week 8 (+/- 3 days)	Week 12 (+/- 3 days)	Week 16 (+/- 3 days)	Week 20 (+/- 3 days)	Week 24 (+/- 3 days)	Early Study Drug Discontinuation	30 Day Follow-Up
Laboratory Assessments												
Viral hepatitis B and C	X											
Chemistry	X						X			X	X	X
CBC with differential, reticulocyte count	X	X		X	X	X	X	X	X	X	X	X
Thiamine status		X					X			X		
Erythropoietin		X				X			X			
C-reactive protein	X	X		X			X			X	X	
Urinalysis	X				X	X	X	X	X	X	**X**	X
Serum pregnancy test[a]	X											
Urine pregnancy test		X			X	X	X	X	X	X	X	
Biomarker Samples												
Bone marrow biopsy & aspirate		X								X		
Buccal swab		X										
Spleen & LIC MRI		X								X	X	
Iron studies	X	X		X	X	X	X	X	X	X		
Hepcidin		X	X	X	X	X	X	X	X	X		
JAK2V617F allele burden and other mutation tests		X								X	X	
Pharmacodynamics[c,d]			X[d]		X					X		
Pharmacokinetics[e]			X		X					X		

Assessment	Screening (-28 to -21 days)	Baseline (-7 +/- 2 days)	Enrollment	Week 2 (+/- 3 days)	Week 4 (+/- 3 days)	Week 8 (+/- 3 days)	Week 12 (+/- 3 days)	Week 16 (+/- 3 days)	Week 20 (+/- 3 days)	Week 24 (+/- 3 days)	Early Study Drug Discontinuation	30 Day Follow-Up
Cytokines/chemokines	X		X[f]		X	X	X	X	X	X		
Exploratory signaling (mass cytometry)		X			X		X			X		
$CD34^+$ cell count	X		X				X		X			
Gene expression	X		X				X		X			
Immunophenotype (flow cytometry)	X		X				X		X			

a For female subject post-menopausal for less than two years, if FSH < 40 mIU/mL a serum pregnancy test will be required
b Hepcidin sample collection will occur at the following times?: baseline visit, between 8am-10am, 6 hours later; enrollment, Weeks 4, 8, 12, 16, 20 and 24 predose (between 8am and 10am) and 6 hours postdose
c Pharmacodynamics biomarker sample collection will occur at the following times: predose, 2,4 and 6 hours postdose at enrollment, then Week 4 and 24.
d Additional exploratory pharmacodynamics sample collection will occur at the following tines: predose, and 2 hours postdose at enrollment only.
e PK sample collection at the following times: predose, 6 hours postdose. All other time points: predose only.
f Cytokine sample collection times at enrollment: predose, 6 hours postdose. All other time points: predose only.
g MMB accountability ONLY at Week 24 and ESDD visits.

Appendix 3. Peripheral Neuropathy SMQ AEs

Name	Code	Level	Scope
Acute painful neuropathy of rapid glycaemic control	10072909	PT	Narrow
Acute polyneuropathy	10066699	PT	Narrow
Amyotrophy	10002027	PT	Narrow
Autoimmune neuropathy	10070439	PT	Narrow
Axonal neuropathy	10003882	PT	Narrow
Biopsy peripheral nerve abnormal	10004846	PT	Narrow
Decreased vibratory sense	10067502	PT	Narrow
Demyelinating polyneuropathy	10061811	PT	Narrow
Guillain-Barre syndrome	10018767	PT	Narrow
Ischaemic neuropathy	10051307	PT	Narrow
Loss of proprioception	10057332	PT	Narrow
Miller Fisher syndrome	10049567	PT	Narrow
Multifocal motor neuropathy	10065579	PT	Narrow
Myelopathy	10028570	PT	Narrow
Nerve conduction studies abnormal	10029175	PT	Narrow
Neuralgia	10029223	PT	Narrow
Neuritis	10029240	PT	Narrow
Neuronal neuropathy	10071579	PT	Narrow
Neuropathic muscular atrophy	10075469	PT	Narrow
Neuropathy peripheral	10029331	PT	Narrow
Notalgia paraesthetica	10072643	PT	Narrow
Peripheral motor neuropathy	10034580	PT	Narrow
Acute painful neuropathy of rapid glycaemic control	10072909	PT	Narrow
Acute polyneuropathy	10066699	PT	Narrow
Amyotrophy	10002027	PT	Narrow
Angiopathic neuropathy	10079036	PT	Narrow
Anti-myelin-associated glycoprotein associated polyneuropathy	10078324	PT	Narrow
Autoimmune neuropathy	10070439	PT	Narrow
Axonal neuropathy	10003882	PT	Narrow
Biopsy peripheral nerve abnormal	10004846	PT	Narrow
Decreased vibratory sense	10067502	PT	Narrow
Demyelinating polyneuropathy	10061811	PT	Narrow

Name	Code	Level	Scope
Guillain-Barre syndrome	10018767	PT	Narrow
Ischaemic neuropathy	10051307	PT	Narrow
Loss of proprioception	10057332	PT	Narrow
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Neuralgia	10029223	PT	Narrow
Neuritis	10029240	PT	Narrow
Neuronal neuropathy	10071579	PT	Narrow
Neuropathic muscular atrophy	10075469	PT	Narrow
Neuropathy peripheral	10029331	PT	Narrow
Notalgia paraesthetica	10072643	PT	Narrow
Peripheral motor neuropathy	10034580	PT	Narrow
Peripheral nervous system function test abnormal	10034591	PT	Narrow
Peripheral sensorimotor neuropathy	10056673	PT	Narrow
Peripheral sensory neuropathy	10034620	PT	Narrow
Polyneuropathy	10036105	PT	Narrow
Polyneuropathy chronic	10064135	PT	Narrow
Polyneuropathy idiopathic progressive	10036111	PT	Narrow
Radiation neuropathy	10068886	PT	Narrow
Sensorimotor disorder	10062162	PT	Narrow
Sensory disturbance	10040026	PT	Narrow
Sensory loss	10040030	PT	Narrow
Small fibre neuropathy	10073928	PT	Narrow
Tick paralysis	10077336	PT	Narrow
Toxic neuropathy	10067722	PT	Narrow

Appendix 4. Cataract MST AEs

MEDDRA Term Name	MEDDRA Code	MEDDRA Level
Atopic cataract	10069649	PT
Cataract	10007739	PT
Cataract congenital	10007747	PT
Cataract cortical	10007748	PT
Cataract diabetic	10007749	PT
Cataract nuclear	10007759	PT
Cataract operation	10063797	PT
Cataract subcapsular	10007764	PT
Cataract traumatic	10007766	PT
Lens discolouration	10070549	PT
Lenticular opacities	10024214	PT
Radiation cataract	10037756	PT
Toxic cataract	10044135	PT
Bioptic eye surgery	10078599	PT
Eye opacity	10078394	PT
Cornea verticillata	10077604	PT

Appendix 5. Leukemic Transformation MST AEs

MEDDRA Term Name	MEDDRA Code	MEDDRA Level
Acute megakaryocytic leukaemia	10000860	PT
Acute megakaryocytic leukaemia (in remission)	10057194	PT
Acute monocytic leukaemia	10000871	PT
Acute monocytic leukaemia (in remission)	10000872	PT
Acute myeloid leukaemia	10000880	PT
Acute myeloid leukaemia (in remission)	10000881	PT
Acute myeloid leukaemia recurrent	10059034	PT
Acute myelomonocytic leukaemia	10000890	PT
Acute promyelocytic leukaemia	10001019	PT
Erythraemic myelosis (in remission)	10015246	PT
Erythroleukaemia	10015281	PT
Acute biphenotypic leukaemia	10067399	PT
Acute leukaemia	10000830	PT
Acute leukaemia in remission	10060930	PT
Acute undifferentiated leukaemia	10073479	PT
Blast cell crisis	10053747	PT
Blast crisis in myelogenous leukaemia	10050282	PT
Chloroma	10008583	PT
Chloroma (in remission)	10008584	PT
Eosinophilic leukaemia	10014958	PT
Leukaemia basophilic	10024293	PT
Leukaemia granulocytic	10024299	PT
Leukaemia monocytic	10024305	PT
Monocytic leukaemia in remission	10061295	PT
Myeloid leukaemia	10028549	PT
Myeloid leukaemia in remission	10061301	PT
Aleukaemic leukaemia	10001660	PT
Central nervous system leukaemia	10066231	PT
Leukaemia	10024288	PT
Leukaemia cutis	10053180	PT
Leukaemia in remission	10061220	PT
Leukaemia recurrent	10062489	PT

MEDDRA Term Name	MEDDRA Code	MEDDRA Level
Leukaemic cardiac infiltration	10077563	PT
Leukaemic infiltration	10069360	PT
Leukaemic infiltration extramedullary	10067117	PT
Leukaemic infiltration gingiva	10067431	PT
Leukaemic infiltration hepatic	10058671	PT
Leukaemic infiltration ovary	10075853	PT
Leukaemic infiltration pulmonary	10052368	PT
Leukaemic infiltration renal	10069359	PT
Leukaemic retinopathy	10059239	PT
Mastocytic leukaemia	10056450	PT
Neonatal leukaemia	10028958	PT

Appendix 6. Validation document of hepcidin testing from Radboud University

Method name	Hepcidine in serum/plasma met WCX-TOF-massaspectrometrie methode		
Work Unit	Iron metabolism		
Method Sop no.	036977	In production	May 2012
Equipment	Microflex LT.	Equipment Sop nr.	032496
Authors	Name	sign	Date
Research technicians	Coby Laarakkers BSc. Erwin Wiegerinck BSc.	Qportal	digital
Supervisor	Prof. Dr. Dorine Swinkels, MD/PhD	Qdoc	digital

ID	*042010 v 3*
Datum print	
Paraaf KAM-adviseur	

Index

1. Generally.

1.1 Purpose.

Purpose is to validate the Weak Cation Exchange -Time Of Flight – Mass Spectrometry (WCX-TOF-MS) method for hepcidin-25 in serum/plasma

1.2 Background.

Hepcidin is an iron regulating peptide hormone and mainly produced in the liver. There are 3 isoforms hepcidin-20 (20 amino-acids [aa]), hepcidin-22 (22 aa) and hepcidin-24(24 aa) hep24 has recently discovered in samples from kidney patients. Only hepcidin-25(25 aa) has been shown to participate in the regulation of iron metabolism. It is thought to be the major regulator of dietary iron absorption and cellular iron release. It exerts its regulatory function by counteracting the function of ferroportin, the major cellular iron exporter in the membrane of macrophages, hepatocytes and the basolateral site of enterocytes. Hepcidin-25 induces the internalization and degradation of ferroportin, resulting in increased intracellular iron stores, decreased dietary iron absorption and decreased circulating iron concentrations. Several physiologic and pathologic processes regulate the synthesis of hepcidin. Iron deficiency, hypoxia, anemia and HFE-related hemochromatosis lead to a decrease in hepcidin synthesis and subsequent increased iron absorption from the intestine. Infection and inflammation lead to an increase in circulating hepcidin levels and subsequent decrease of iron absorption from the intestine and iron entrapment in the reticulo-endothelial macrophages, which may result in anemia of chronic disease. See for more information: www.hepcidinanalysis.com and (1, 2,8).
Since 2005 we measure hepcidin in our laboratory which resulted in numerous publications in peer reviewed journals.
In the clinic this assay is currently used as a diagnostic or monitoring tool for unexplained i) iron overload or ii) iron deficiency anemia. Moreover, the assay is exploited for clinical studies aiming for a better understanding of the pathophysiology of iron disorders and clinical trials in the monitoring or treatment with newly developed hepcidin modulating agents (11).

1.3 Responsibilities.

Performance:	Coby Laarakkers, BSc.
	Erwin Wiegerinck BSc.
Supervision:	Prof. Dr. Dorine Swinkels, MD/ PhD.
End responsibility	Prof. Dr. Dorine Swinkels, MD/ PhD.

1.4 Evaluation period.

Start date:	October 2011.
Scheduled end date:	Mai 2012

2. Equipment/ method/ assay.

Equipment:	Microflex LT.
Method:	WCX-TOF-MS.
Assay:	Hepcidin-25 in serum.

3. Definition and Terminology.

3.1 Abbreviations.

WCX	-Weak Cation Exchange.
TOF	-Time Of Flight.
MS	-Mass Spectrometry.

3.2 Units and decimals.

Assay	Unit	Significant figures
Hepcidin-25	nM	3

4. Procedure.

4.1 Internal standard heavy hepcidin-25

Procedure.
For this assay we have a custom made heavy hepcidin-25 internal standard (mass 2829.4 Da); DTHF ($^{13}C_9$,^{15}N)P($^{13}C_5$,^{15}N)I($^{13}C_6$,^{15}N)CI($^{13}C_6$,^{15}N)F($^{13}C_9$,^{15}N)CCG(^{15}N)CCHRSKCGMCCKT disulfide bridged (Peptide International, AF-179, Lot No221-006221)(9).
10 vials, freeze dried heavy hepcidin-25 are solved in H_2O in accordance with the manual. Solved heavy hepcidin25+40 of all the 10 vials were put together and mixed. Heavy hepcidin-25 with a concentration of 0.1 mM is aliquoted á 12 µL in PCR tubes á 200 µL and stored at -80ºC in deep freezer "Iron metabolism" room 4.07 M325.04.264.
This internal standard is controlled by 10 x measurement of HiQC and LoQC.
The average of these 10 measurements should not differ more than 0.2 nM of the nominal values of the controls.
Data: H\microflex\2 controle IS hep25+41.
HiQC 2014: 10.12 ± 0.2 nM
LoQC 2014: 2.34 ± 0.1 nM

4.2 Calibration hepcidin mass.

Procedure.
Calibration of the Microflex LT will be performed after ion source cleaning and services.
The calibrator contains hepcidin-20, hepcidin-22, hepcidin-24 (Peptides International, custom made, PCS-37242-PI, synthetic) and hepcidin-25 (Peptides International, PLP-4392-s, synthetic). Hepcidin-20 and 22 are a special gift from Tom Ganz and are isolated from urine (3). The mass values of the hepcidins can be found at UniProtKB (Proteinknowledgebase).

Peptide	**m/z**
Hepcidin-20	2191.77
Hepcidin-22	2436.06
Hepcidin-24	2673.90
Hepcidin-25	2789.40

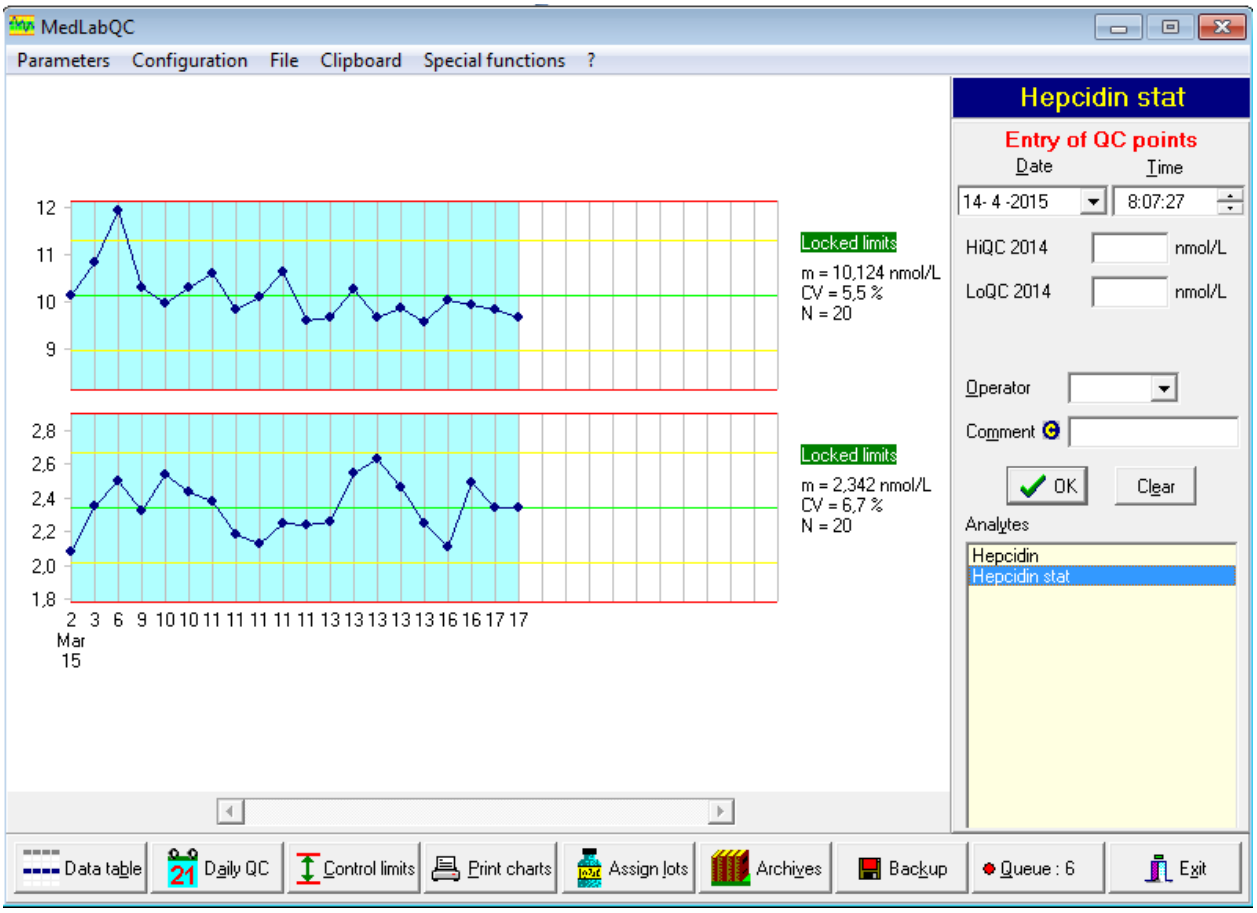
4.3 Internal Quality Control and Precision.

Procedure.
Precision is calculated by using MedLabQC (version:Philippe Marquis 3.24)
Control charts show the internal quality control. Hepcidin-25 results for the QC controls for the subsequent measurement runs.

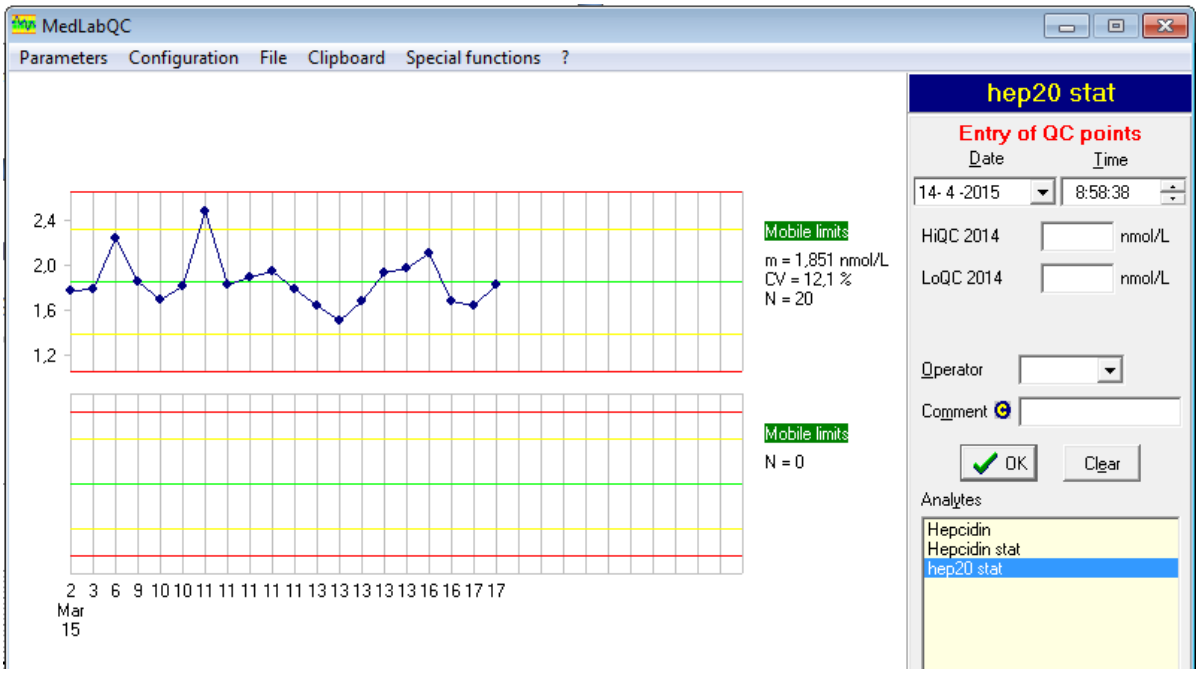
Material.
HiQC 2014: heparin plasma pool is made by pooling samples from intensive care patients and plasma from an iron depleted HFE-hemochromatosis patient.
LoQC 2014: heparin plasma pool is made by pooling samples from 3 iron depleted hemochromatosis patients and plasma from intensive care patients.
The controls are aliquoted in polypropylene tubes (2 mL) á 250 ul sample. In deep freezer (-80 ˚C) "Iron metabolism" room 4.07 M325.04.264. number. For both QC's there are about 500 samples in the freezer.

4.3.1 Results MedLabQC

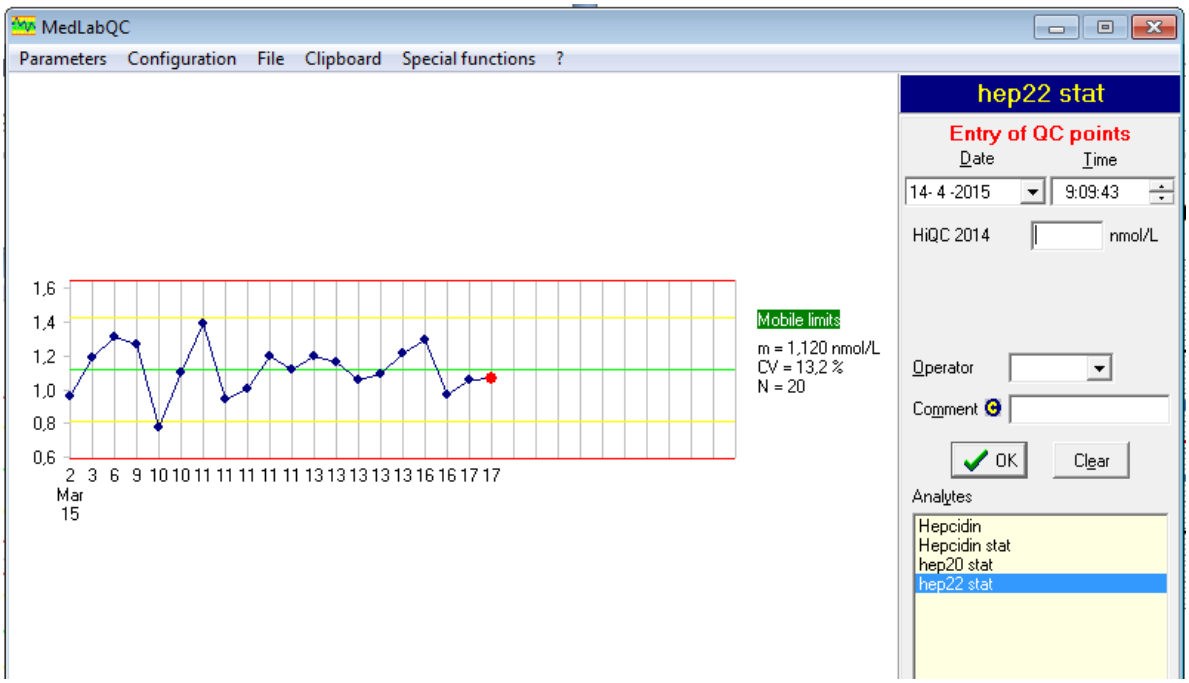
HiQC and LoQC 2014
Hepcidin-25 measurements March 2015



HiQC 2014
Hepcidin-20 measurements March 2015

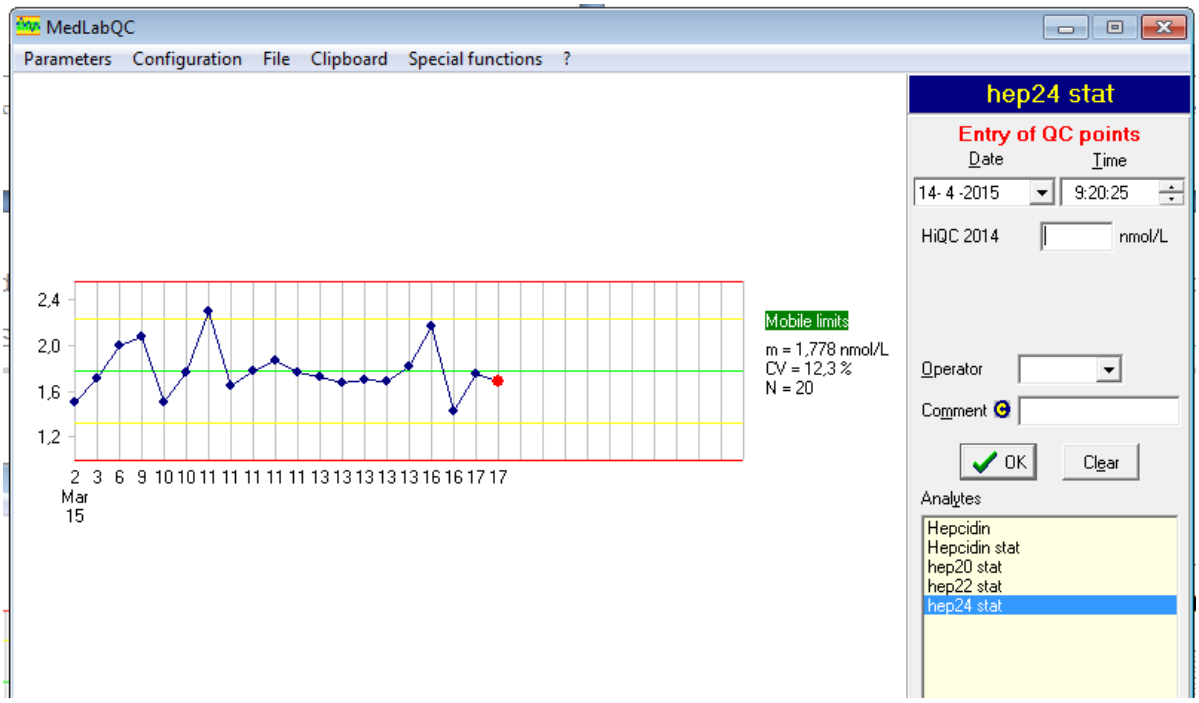


HiQC 2014
Hepcidin-22 measurements March 2015



HiQC 2014

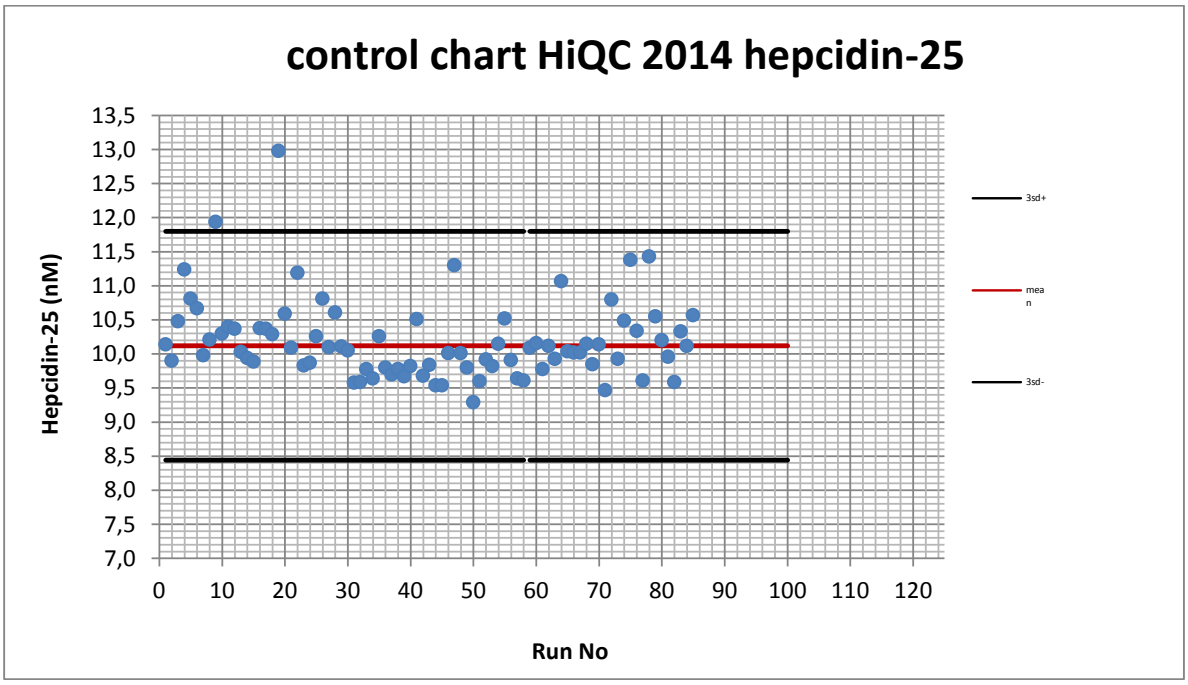
Hepcidin-24 measurements March 2015



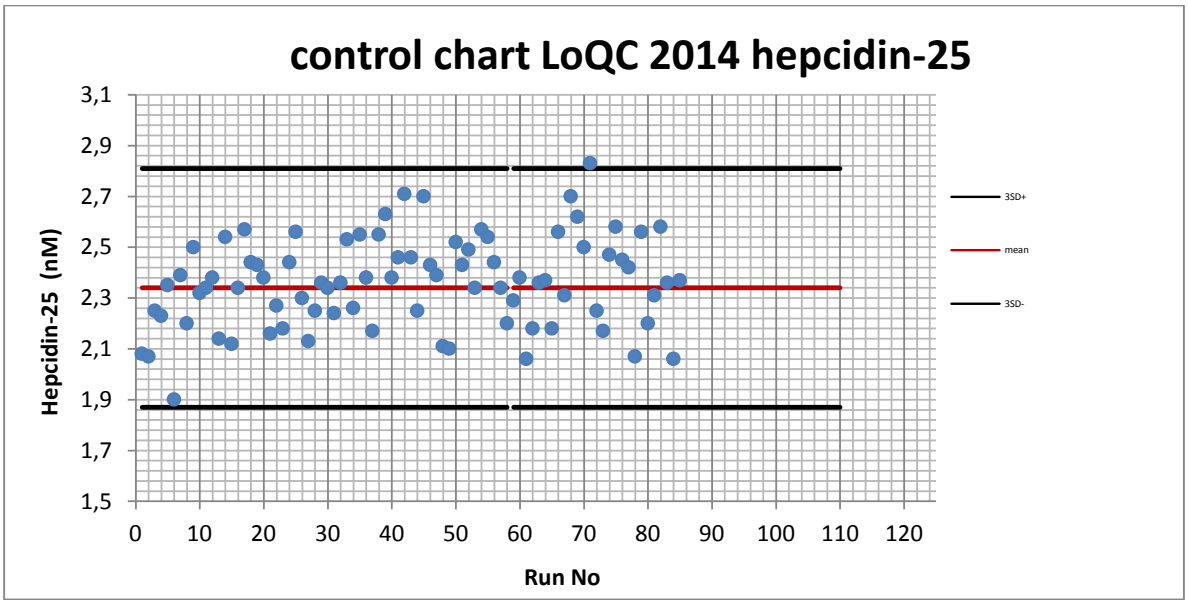
	HiQC 2014	LoQC 2014
	Hep25	
n	20	20
average	10.12 nM	2.34 nM
SD	0.557 nM	0.157 nM
3x SD	1.67 nM	0.47 nM
CV	5.5 %	6.7 %

	HiQC 2014		
	Hep20	**Hep22**	**Hep24**
n	20	20	20
average	1.85 nM	1.12 nM	1.78 nM
SD	0.224 nM	0.148 nM	0.219 nM
3x SD	0.67 nM	0.44 nM	0.66 nM
CV	12.1 %	13.2 %	12.3%

4.3.2 Control charts HiQC and LoQC hepcidin-25



Hepcidin-25 measurements March –April 2015.



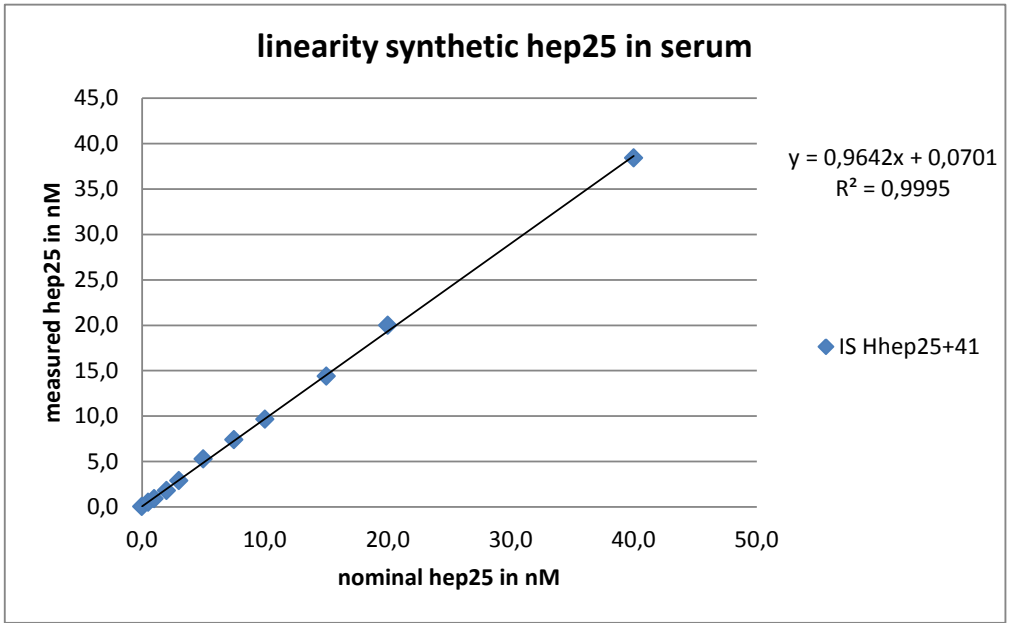
Hepcidin-25 measurements March –April 2015

Control charts: H\LKN\ijzermetabolisme\Microflex\1-controle kaarten

4.4 Linearity curve synthetic Hepcidin-25.

Procedure.
For this curve we used zero hepcidin serum coming from patient with juvenile haemochromatosis due to a homozygous mutation in the hemojuvelin gene (4). Different concentrations synthetic hepcidine-25 are added to this serum.
.

A. Linearity curve range (0-40 nM): concentrations synthetic hepcidine-25 added: 0, 0.5,1, 2, 3, 5, 7.5, 10, 15, 20 en 40 nM.



Linearity curve (0- 40.0 nM) synthetic hepcidin-25 in zero serum with heavy hep25 as internal standard.

Conclusion.
The curve is linear to at least 40 nM.

4.5 Intra run.

Procedure.
4 patient plasma samples were measured 8 times in one run with WCX-TOF-ms. method with the Microflex LT.

Results (hepcidin-25 in nM).

n=8	1	2	3	4
average	10.34	2.59	3.52	7.40
STD	0.22	0.15	0.12	0.26
CV (%)	2.14	2.67	3.31	3.53

3 of these 4 samples contained isoforms of hepcidin-25 (> 1 nM)

Results (hepcidin-20 in nM).

n=8	1	3	4
average	2.34	5.75	4.90
STD	0.18	0.35	0.21
CV (%)	7.61	6.04	4.20

Results (hepcidin-22 in nM)

n=8	1	4
average	1.36	1.71
STD	0.12	0.13
CV (%)	8.62	7.87

Results (hepcidin-24 in nM)

n=8	1	3	4
average	2.25	1.38	2.37
STD	0.09	0.11	0.07
CV (%)	4.01	8.22	2.89

Conclusie.
Intra run CV for hepcidin-25: < 3.6%.
Intra run CV's for the hepcidin isoforms are higher (between 2.9 and 8.6 %).

4.6 Inter run.

Procedure.
Two plasma samples are measured on 8 different days (in 8 different runs) with the WCX-TOF-ms method for hepcidin-25 on Microflex LT.

Results (hepcidin-25 in nM).

n=8	5	6
average	10.47	2.58
STD	0.49	0.21
CV (%)	4.63	8.26

Results (hepcidin-20, 22, 24 in nM)

n=8	Sample 5 Hep20	Sample 5 Hep22	Sample 5 Hep24
average	2.50	1.38	2.31
STD	0.34	0.16	0.39
CV (%)	13.7	11.6	16.8

Conclusie.

Interrun hepcidin-25: CV< 8.3 %
Interrun hepcidin-isoforms: CV: 11.6-16.8 %

4.6.1 Linearity of native hepcidin-25 in serum/plasma (1).

Material.
4 serum samples of healthy volunteers are selected for this test. Two samples have a hepcidin-25 concentration above 10 nM and 2 samples a concentration below 3 nM.

Procedure.
We tested linearity (1) in duplicate and used there for 4 serum samples, 2 with a high hepcidin concentration and 2 with a low hepcidin concentration. 1 High and 1 low sample is measured in duplicate in different mixture ratio on Microflex LT. You can find in table below the used mixture ratios. Based on the known values of the undiluted samples we calculated the rate value of the mixtures. The test should be linear for the evaluated range and the measured values should be equal to the calculated values.

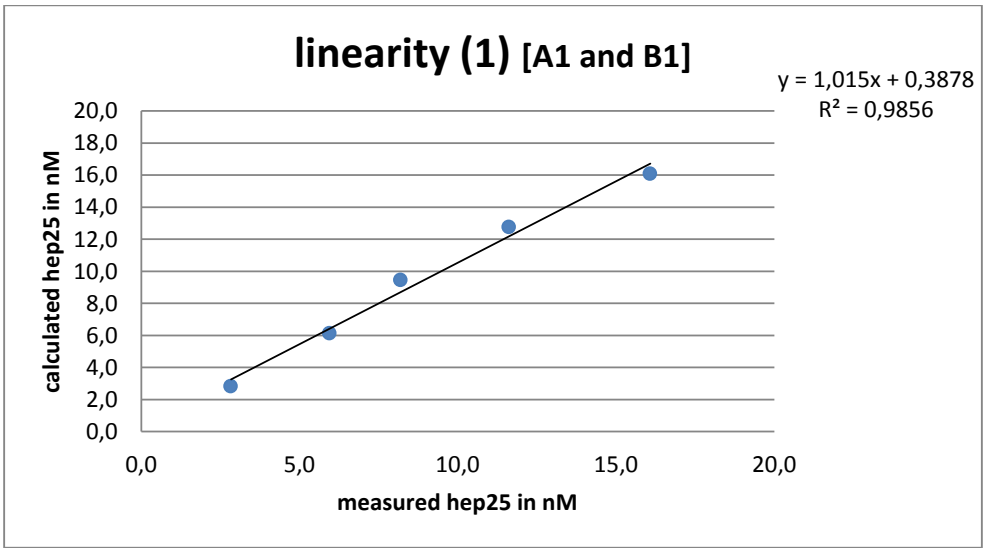
Table: Mixture ratios.

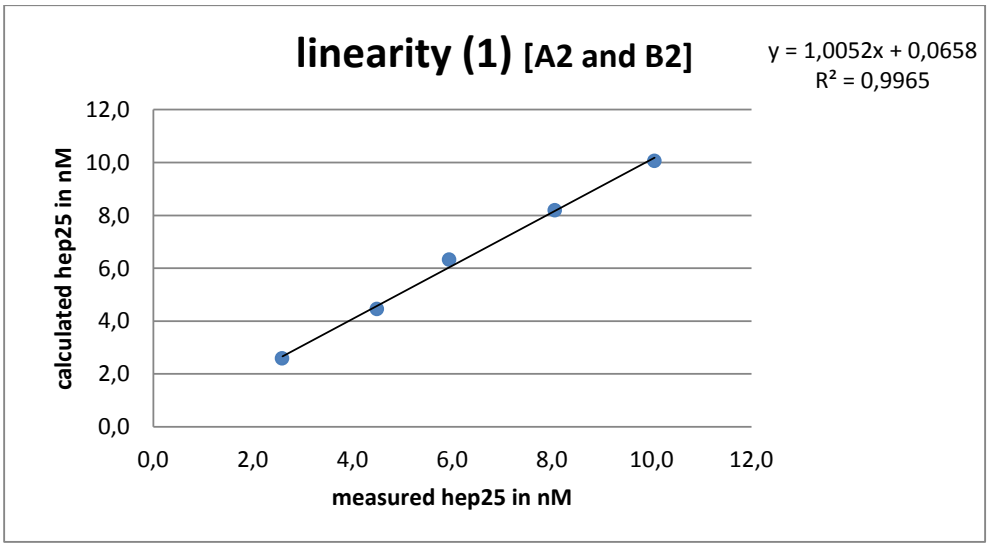
	Sample A (low)		Sample B (high)		Calculated value
1	1 volume	50 µL	nvt	0 µL	= sample A
2	3 volumes	37.5 µL	1 volume	12.5 µL	= (3 x A + 1x B) / 4
3	2 volumes	25 µL	2 volumes	25 µL	= (2 x A + 2 x B) / 4
4	1 volume	12.5 µL	3 volumes	37.5 µL	= (1 x A + 3 x B) / 4
5	na	0 µL	1 volume	50 µL	= sample B

Results (Hepcidin-25 in nM).

Samples A1 and B1	Measured value (avg duplicate)		Calculated value	Difference between measured and calculated value
1	16.1		16.1	0.0
2	11.6		12.8	1.2
3	8.2		9.5	1.3
4	6.0		6.1	0.1
5	2.8		2.8	0.0

Samples A2 and B2	Measured value (avg duplicate)		Calculated value	Difference between measured and calculated value
1	10.1		10.1	0.0
2	8.1		8.2	0.1
3	5.9		6.3	0.4
4	4.5		4.5	0.0
5	2.6		2.6	0.0





Conclusion: the method is linear to at least 16 nM .

4.6.2 **Linearity** of native hepcidin-25 in serum/plasma **(2).**

Procedure.
To test linearity (2) we used 2 samples. These samples were measured in 5 different dilutions see table below and measured in duplicate on Microflex LT.

Table: Dilutions.

	sample
1	undiluted
2	1.25x
3	1.66x
4	2x
5	5x
6	10x

Material.
We selected 1 serum sample from a healthy volunteer (CH2) with a hepcidin concentration above 10 nM and 1 plasma sample from a kidney patient (KF218) with a high hepcidin value. See for dilutions table above.

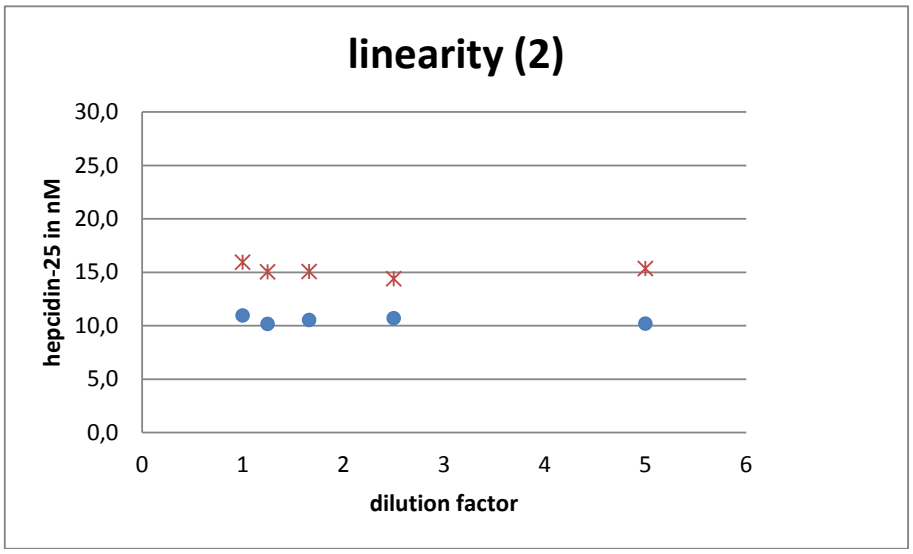
Results (hepcidin-25 in nM).

	KF218	CH2
	Hep25 in nM	
undiluted	15.9	11.0
1.25x	12.0	8.1
1.66x	9.1	6.3
2.5x	5.8	4.3
5x	3.1	2.0

Measured hepcidin.

Dil.factor	KF218	CH2
	Hep25 in nM	
1	15.9	11.0
1,25	15.0	10.2
1,66	15.1	10.5
2,5	14.4	10.7
5	15.4	10.2
avg	15.15	10.51
sd	0.507	0.302
VC	3.35%	2.87%

Calculated hepcidin levels using the measured value and the dilution factor.



Measured hepcidin-25 values: stars = HF218; dots = CH2

Conclusion.
Hepcidin measurement in these samples is linear for at least to 15 nM.

4.7 Detection limit

The detection limit is determined at 0.5 nM. The signal/noise ratio was >2 for a zero serum added with 0.5 nM synthetic hepcidin-25 (5).

4.8 Reference values.

Procedure.

The reference range of serum hepcidin-25 is <0.5 nM-14.7 nM (median 4.5 nM) for men, <0.5 -12.3 nM (median 2.0 nM) for premenopausal women, and <0.5-15.6 nM (median 4.9 nM) for postmenopausal women. We have also assessed i) references ranges per 5-year age group for men and women; ii) for hepcidin/ferritin ratio and transferrin saturation/hepcidin ratio. These reference levels for the WCX-TOF MS method are derived from those of our ELISA method[6,7], based on the regression line between the ELISA and WCX-TOF MS results obtained for the same samples from patients without hepcidin isoforms[1,7].
We also assessed:

- references ranges for serum hepcidin-25 and hepcidin/ferritin ratio in children (6 months to 3 years old) in the normative population (N=292) as measured by weak cation exchange time-of-flight mass spectrometry (WCX-TOF MS) [9].
- Reference ranges for serum hepcidin-25 during pregnancy [12].

More details on the WCX-TOF MS reference values for hepcidin can be found on our website www.hepcidinanalysis.com and in the references.

4.9 measurement uncertainty

The measurement uncertainty is 14.1%, calculated by $CV_{\text{total variation analyse}}$
This is lower than $½CV_{\text{biological variation}}$
Calculation see supplement 1

Results:

MS-method_ hepcidin-25 (nM)

Reference ranges for serum hepcidin (nM) per 5-year age group for adult men and women in the reference population.

	Men (N=1,066)					Women (N=882)				
				95% reference range					95% reference range	
Age, years	N	(%)	Median	P2.5	P97.5	N	(%)	Median	P2.5	P97.5
18-24	10	(1)	5.3	0.8	11.0	21	(2)	1.1	< 0.5	6.3
25-29	16	(2)	4.9	< 0.5	15.3	28	(3)	1.4	< 0.5	6.6
30-34	18	(2)	4.2	< 0.5	15.8	24	(3)	1.9	< 0.5	13.1
35-39	22	(2)	3.6	< 0.5	12.1	36	(4)	1.5	< 0.5	9.9
40-44	19	(2)	6.1	< 0.5	11.0	65	(7)	2.5	< 0.5	15.2
45-49	76	(7)	3.4	< 0.5	13.2	110	(12)	1.6	< 0.5	8.9
50-54	106	(10)	4.0	< 0.5	13.8	140	(16)	2.9	< 0.5	14.3
55-59	173	(16)	4.4	< 0.5	15.7	129	(15)	4.9	< 0.5	13.6
60-64	179	(17)	4.5	< 0.5	14.3	137	(16)	4.7	< 0.5	17.3
65-69	186	(17)	5.3	< 0.5	13.9	95	(11)	4.9	< 0.5	14.2
70-74	133	(12)	4.9	< 0.5	17.0	62	(7)	5.1	< 0.5	24.2
75-79	99	(9)	3.8	< 0.5	16.1	16	(2)	5.4	0.8	18.4
80-84	22	(2)	3.8	1.6	12.6	10	(1)	7.2	< 0.5	12.0
≥85	7	(1)	6.8	1.6	12.8	9	(1)	3.7	< 0.5	15.4
All	1,066	(100)	4.5	< 0.5	14.7	882	(100)	3.6	< 0.5	14.6

Premenopausal women (<55 year): (N=424)
- Median = 2.0 nM
- P2.5 = 0.1 nM (<0.5 nM)
- P97.5 = 12.3 nM

Postmenopausal women (>=55 year): (N=458)
- Median = 4.9 nM
- P2.5 = 0.2 nM (<0.5 nM)
- P97.5 = 15.6 nM

Reference ranges for serum hepcidin (nM) in children aged 6 months to 3 years.

Hepcidin		95% CI	
N	Median	P2.5	P97.5
292	3.6	0.94	12.2

Hepcidin				95% CI	
Age (months)	N	(%)	Median	P2.5	P97.5
3-12	111	(38)	4.1	1.1	12.8
13-18	62	(21)	3.8	0.8	12.0
19-24	43	(15)	2.5	0.9	11.8
25-30	38	(13)	3.4	0.8	21.7
31-36	38	(13)	3.9	0.3	14.6

Hepcidin			95% CI	
Gender	N	Median	P2.5	P97.5
Male	207	3.6	0.9	11.9
Female	85	4.0	0.8	14.0

Hepcidin			95% CI	
Time of sample collection	N	Median	P2.5	P97.5
<12.00 pm	170	3.0	0.9	11.4
12.00-17.00 pm	122	4.5	1.1	14.0

Reference range for serum hepcidin in specified time periods during pregnancy

Gestation			95 % reference range	
weeks	**N**	**Median**	**P2.5**	**P97.5**
7-15	**22**	**1.9**	**< 0.5**	**8.6**
19-25	**21**	**< 0.5**	**< 0.5**	**6.8**
29-35	**16**	**< 0.5**	**< 0.5**	**2.4**

Hepcidin-25/ferritin (pmol/µg)

Reference ranges for hepcidin/ferritin per 5-year age group for adult men and women in the reference population.

Men: (N=1064)
- Median = 26.7 pmol/µg
- P2.5 = 2.9 pmol/µg
- P97.5 = 87.9 pmol/µg

Premenopausal women (<55 year): (N=424)
- Median = 35.7 pmol/µg
- P2.5 = 3.0 pmol/µg
- P97.5 = 167.3 pmol/µg

Postmenopausal women (>=55 year): (N=458)
- Median = 40.5 pmol/µg
- P2.5 = 9.1 pmol/µg
- P97.5 = 143.1 pmol/µg

Reference ranges for hepcidin/ferritin in children aged 6 months to 3 years.

MS-method_ TSAT/hepcidin-25 (%/nM)

Men: (N=1059)
- Median = 7.3 %/nM
- P2.5 = 1.7 %/nM
- P97.5 = 256.3 %/nM

Premenopausal women (<55 year): (N=422)
- Median = 13.9 %/nM
- P2.5 = 2.0 %/nM
- P97.5 = 330.0 %/nM

Postmenopausal women (>=55 year): (N=457)
- Median = 5.7 %/nM
- P2.5 = 1.5 %/nM
- P97.5 = 73.4 %/nM

5. Clinical validation.

Zie www.hepcidinanalysis.com/expertise/ (1).

6 Archivating

Word-document: H-LKN\ijzermetabolisme\microflex\kwaliteit\validatie rapport hep25
Laboratory notebook: 485

7. References.

1. http://www.hepcidinanalysis.com/

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11. van Eijk LT, John AS, Schwoebel F, Summo L, Vauléon S, Zöllner S, Laarakkers CM, Kox M, van der Hoeven JG, Swinkels DW, Riecke K, Pickkers P. Effect of the antihepcidin Spiegelmer lexaptepid on inflammation-induced decrease in serum iron in humans. Blood. 2014;124(17): 2643-6.

12. van Santen S, Kroot JJ, Zijderveld G, Wiegerinck ET, Spaanderman ME, Swinkels DW. The iron regulatory hormone hepcidin is decreased in pregnancy: a prospective longitudinal study. Clin Chem Lab Med 2012; 14: 1-7.

Invoer referentiewaardes	HELIX / GLIMS*
Naam invoerder	Nvt (geen wijzigingen)
Datum	
Handtekening	

* = Doorhalen wat niet van toepassing is

Supplement 1

Bijlage meetonnauwkeurigheden.

Biologische variatie$_{\text{tussen dag}}$ humaan hepcidin is 48.8% (Murphy et al[1])

$CV_{\text{totale variatie (pre)-analyse}} < ½\ CV_{\text{biologische variatie}}$

Berekening:

$$CV_{\text{totale variatie (pre)-analyse}} = \sqrt{(CV_{\text{analyse}})^2 + (CV_{\text{pre-analyse 24h}})^2}$$

$$CV_{\text{totale variatie (pre)-analyse}} = \sqrt{(10)^2 + (10)^2}$$

$$CV_{\text{totale variatie (pre)-analyse}} = \sqrt{200}$$

$$CV_{\text{totale variatie (pre)-analyse}} = 14.1\%$$

$CV_{\text{totale variatie (pre)-analyse}} < ½\ CV_{\text{biologische variatie}}$

14.1% < ½ x 48.8% → pass

Serum en plasma samples mogen een dag bij KT blijven staan.
Afspraak is samples dezelfde dag nog in de vriezer.

1. Murphy et al, Quantitation of hepcidin from human and mouse serum using liquid chromatography tandem mass spectrometry, Blood, Vol 110, 3.
2. The Hitch-hiker's Guide to Measurement Uncertainty (MU) in Clinical LaboratoriesApril 2012
3 Graham White, SA Pathology, Flinders Medical Centre, Bedford Park, Adelaide, SA 5042, Australia
4 Clin Chem 2011 Sep 57(9): 1334-6. Gross overestimation of total allowable error based on biological variation. Oosterhuis WP

Appendix 7. Validation document of pSTAT testing from Covance

LabCorp
CLINICAL TRIALS

Client Acceptance of Report

Sponsor: Gilead Sciences
Protocol Title: Detect Phosphorylated STAT3 in IL6 Stimulated Whole Blood (With Additional Preparation and Storage for Future ELISA Assay)
Protocol: GS-US-352-1151 (USA)/1153 (EU)/ others
Report Number: LCT13050-QR01-01

LabCorp Clinical Trials Management has reviewed the data in the validation report described above. An audit of the report was finalized by LabCorp Clinical Trials QA on the date below. Please review the report and provide a signature accepting the report and the data within.

Client has 10 business days from date signed by LCT QA below to declare acceptance of report. If no response is received within 10 business days the report is considered final and will be archived internally.

K. DeMarco Morgan — 12-Dec-2014

R. DeMarco Morgan/QA Auditor
LabCorp Clinical Trials

Date

Kevin Kwei — 30-Oct-2015

~~Anita Reddy~~ Kevin Kwei
Gilead Sciences

Date

Comments: Sent to Anita Reddy (Anita.Reddy@gilead.com) on 12-Dec-2014. RDM
12-Dec-2014

Document forwarded on CD to Anita Reddy on 14 Dec 2014.
LBJ
14 Dec 2014

M.s. Reddy acknowledged receipt on 18 Dec 2014.
RBJ
18 Dec 2014

Assay Transfer and Qualification of a Custom Flow Cytometric Assay to Detect Phosphorylated STAT3 in IL6 Stimulated Whole Blood (With Additional Preparation and Storage for Future ELISA Assay)

Flow Cytometry Performed by:
LabCorp Clinical Trials
201 Summit View Drive, Suite 200
Brentwood, TN 37027

Conducted for:
Gilead Science, Inc.
368 Lakeside Dr.
Foster City, CA 94404

Validation Initiation Date: January 2014
Validation End Date: April 2014

LabCorp Clinical Trials Review:

Kenneth Pennline, Ph.D.
Vice President and Global Head, Cytometry Services
LabCorp Clinical Trials – Brentwood

Date 04-Dec-2014

Leanne Flye
Manager II – Advanced Cytometric Applications
LabCorp Clinical Trials – Brentwood

Date 03 Dec 2014

Rachael Wilson
Supervisor – Advanced Cytometric Applications
LabCorp Clinical Trials – Brentwood

Date 03 Dec 2014

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LIST OF EQUATIOINS

LabCorp
CLINICAL TRIALS

Quality Assurance Statement—Good Clinical Practice

Sponsor:	Gilead Sciences, Inc.
Protocol Number:	GS-US-352-1151 (USA)/1153 (EU)/ others
Protocol Title:	Assay Transfer and Qualification of a Custom Flow Cytometric Assay to Detect Phosphorylated STAT3 in IL6 Stimulated Whole Blood (With Additional Preparation and Storage for Future ELISA Assay)

The clinical laboratory study was audited according to the Standard Operating Procedures of LabCorp Clinical Trials.

The following attests to the dates that audits were conducted and to the findings reported:

Date(s) of Audit	Type of Audit	Date Reported to LabCorp Clinical Trials Management
21May2014 – 12Jun2014	**Qualification Data Audit**	**12Jun2014**
05Dec2014	**Assay Transfer Data Audit**	**05Dec2014**
12Jun2014	**Report Audit**	**12Jun2014**

R. DeMarco Morgan

R. DeMarco Morgan/QA Auditor
LabCorp Clinical Trials

05-Dec-2014
Date

BACKGROUND

The project involved the assay transfer of an assay that had been developed internally at Gilead Sciences, Inc. Subsequent qualification included reproducibility and linearity experiments, but did not include stability, as that was assessed by client. The work herein qualified the assay for the measurement of pSTAT3 levels in CD3+ and CD3+/CD4+ lymphocytes in healthy donor peripheral whole blood that had been stimulated with Interleukin-6 for use in clinical trials.

ASSAY DESCRIPTION AND REPORTABLES

The panel and associated reportables for the validation include the following:

Gilead pSTAT3 Stimulation Assay Panel

	Panel			
Conditions	Tube	FITC	PE	V450
Unstimulated-Tube 1	FMO-PE	CD3	blank	CD4
Unstimulated-Tube 2	Total STAT3	CD3	Total STAT3	CD4
Unstimulated-Tube 3	pSTAT3	CD3	pSTAT3	CD4
IL-6 Stim Tube 4	FMO-PE	CD3	blank	CD4
IL-6 Stim Tube 5	Total STAT3	CD3	Total STAT3	CD4
IL-6 Stim Tube 6	pSTAT3	CD3	pSTAT3	CD4

Gilead pSTAT3 Stimulation Assay Reportables

- %Lymphs
- Region Events CD3+ Lymphs
- %CD3+ [Lymphs]
- Region Events CD3+/CD4+ Lymphs
- %CD3+/CD4 [Lymphs]
- MESF FMO-PE unstim [CD3+ Lymphs]
- MESF FMO-PE unstim [CD3+/CD4+ Lymphs]
- %tSTAT3 unstim [CD3+ Lymphs]
- %tSTAT3 unstim [CD3+/CD4+ Lymphs]
- MESF tSTAT3 unstim [CD3+ Lymphs]
- MESF tSTAT3 unstim [CD3+/CD4+ Lymphs]
- %pSTAT3 unstim [CD3+ Lymphs]
- %pSTAT3 unstim [CD3+/CD4+ Lymphs]
- MESF pSTAT3 unstim [CD3+ Lymphs]
- MESF pSTAT3 unstim [CD3+/CD4+ Lymphs]
- MESF FMO-PE STIM [CD3+ Lymphs]
- MESF FMO-PE STIM [CD3+/CD4+ Lymphs]
- %tSTAT3 STIM [CD3+ Lymphs]
- %tSTAT3 STIM [CD3+/CD4+ Lymphs]
- MESF tSTAT3 STIM [CD3+ Lymphs]
- MESF tSTAT3 STIM [CD3+/CD4+ Lymphs]
- %pSTAT3 STIM [CD3+ Lymphs]

- %pSTAT3 STIM [CD3+/CD4+ Lymphs]
- MESF pSTAT3 STIM [CD3+ Lymphs]
- MESF pSTAT3 STIM [CD3+/CD4+ Lymphs]
- MFI FMO-PE unstim [CD3+ Lymphs]
- MFI FMO-PE unstim [CD3+/CD4+ Lymphs]
- MFI tSTAT3 unstim [CD3+ Lymphs]
- MFI tSTAT3 unstim [CD3+/CD4+ Lymphs]
- MFI pSTAT3 unstim [CD3+ Lymphs]
- MFI pSTAT3 unstim [CD3+/CD4+ Lymphs]
- MFI FMO-PE STIM [CD3+ Lymphs]
- MFI FMO-PE STIM [CD3+/CD4+ Lymphs]
- MFI tSTAT3 STIM [CD3+ Lymphs]
- MFI tSTAT3 STIM [CD3+/CD4+ Lymphs]
- MFI pSTAT3 STIM [CD3+ Lymphs]
- MFI pSTAT3 STIM [CD3+/CD4+ Lymphs]

EXPERIMENTS

Phase I: Antibody Titration

This phase was conducted in order to titrate all reagents to saturation within the client method. All single color reagents were titrated within the method on 3 applicable donors (normal whole blood drawn into sodium heparin for surface antibodies, and IL-6 stim/unstim for pSTAT3). Once saturation was determined for each marker, the combined panel was tested on 3 additional donors within the panel. No preparation of ELISA samples was done in this phase.

Phase II: Assay Linearity

In order to determine the minimum number of lymphocytes required to accurately determine the MESF/MFI and the % positivity of the phosphorylated epitopes of interest, the following was done: 3 normal whole blood samples were drawn into sodium heparin and then assayed within the method starting at between 0.5-1x10^6 cells per tube. The samples were acquired using a stop gate on the lymphocyte population (limiting population) for 2000, 1000, 500, 300, 100, and 50 events. These samples were processed in singlet by one operator. Each sample was acquired in triplicate. The 2000 event sample was used as the basis for determining the relative error of the MESF/MFI and the % positivity.

Phase III: Inter-Operator and Intra-Assay Precision

2 normal whole blood specimens were drawn into NaHeparin. The samples were shipped back to Brentwood LCT within 1 Day ex-vivo using the Credo GTC4L, 2-8°C shippers from World Courier. The samples were tested immediately within the panel by 2 operators in triplicate. The acquisition stop gate was set to acquire 2000 lymphocytes per client request for this phase.

Phase IV: Assay Transfer -- ELISA testing only

Reference LA-LCT Assay Qualification Plan GEN82, Cross Laboratory Qualification of Human STAT3 [pY705] ELISA (cell lysate, units/mL)

The Brentwood facility drew 2 NWB donors (6mL NaHeparin tubes, 2 per site) and shipped to each of the processing locations. One 6-mL tube was processed on the same day of receipt and the second 6-mL tube was

processed the next day. Opened sample was discarded after samples have been prepped to ensure that only the sterile, unopened sample is used on Day 2. The 2 vials of blood from each donor were shipped to each LCT Cranford, LCT LA laboratories and to Gilead for further processing. The samples were shipped using World Courier Credo GTC4L, 2-8°C shippers only containing temperature monitors. The specimens were processed in each location on day of receipt by operator 1 (only to lysate point) and again on Day 2 by operator 2 (only to Lysate point). The specimens were processed in quadruplicate (4 stimulated replicates and 4 unstimulated replicates per donor). The Cranford lab prepared double aliquots of each replicate. One set was shipped to LCT-LA for further processing in ELISA; the other set was shipped to Gilead for further processing in ELISA. The LCT-LA lab also processed the samples to the lysate point. One set was retained to complete in the ELISA assay, the other set was shipped back to Gilead for them to complete the ELISA assay. The ELISA runs were performed in triplicate on each lysate replicate by 2 different operators. The Assay Transfer was to be repeated 2 additional times using 2 different normal healthy donor specimens approximately one week after first run. This process was actually repeated on 6 total donors due to variability during runs. Results for the ELISA were compared between Gilead and LCT-LA on these lysates. Additionally, the LCT-LA lab performed the ELISA lysates and performed the entire assay from start to completion on the received whole blood for comparison with Gilead processing from start to completion. Data for all of these comparisons described herein were presented in the corresponding report for Assay Qualification Plan GEN82 and not included, except in conclusion and reference in this report. Acceptance criteria for said ELISA comparison data are also located in Assay Qualification Plan GEN82.

Phase IV: Assay Transfer – Flow Cytometry testing only

The Brentwood facility drew 3 NWB donors and ship to LCT Cranford flow lab using World Courier Credo GCT4L, 2-8°C shippers. The samples were processed according to CT-BW-SS-SOP-398, Gilead Specific Flow Cytometry Assay to Detect STAT3 in IL6 Stimulated Whole Blood, in triplicate by one operator. Mean, Standard deviation and %CVs were calculated between replicates. Percent stimulation was calculated for each donor as well using the following equation:

Equation 1: % stim = (stim – unstim)/unstim x 100

MATERIALS AND METHODS

Gilead pSTAT3 Stimulation Assay Method

The assay was performed according to CT-BW-SS-SOP-398, Gilead Specific Flow Cytometry Assay to Detect STAT3 in IL6 Stimulated Whole Blood.

Reagents

The reagents utilized in the flow cytometric assays are listed below:

- CD3 FITC, clone UCHT1, BD Cat. No. 555916
- CD4 V450, clone RPA-T4, BD Cat No. 560345
- STAT3 PE, clone M59-50, BD Cat No. 560391
- pSTAT3-PE, (Y705), Clone 4/P-STAT3, BD Phosflow Cat No. 612569
- BD Lyse/Fix, BD Cat No. 558049
- BD Perm Buffer III BD PhosFlow Cat No. 558050
- BD Stain Buffer (FBS), BD Cat No. 554656
- Recombinant Human IL-6, R&D Systems Cat No. 206-IL-010
- PBS with 1% BSA – Prepared according to CT-BW-REAG-SOP-14, Preparation of 1X Phosphate-Buffered Saline with 2% Bovine Albumin (PBS-BSA with azide), 1X Phosphate-

- PBS with 1% BSA – Prepared according to CT-BW-REAG-SOP-14, Preparation of 1X Phosphate-Buffered Saline with 2% Bovine Albumin (PBS-BSA with azide), 1X Phosphate-Buffered Saline with 1% Serum Bovine Albumin (PBS with 1% BSA with azide), and 0.5% Serum bovine Albumin (PBS-BSA without azide)
- Ammonium Chloride Based Whole Blood Lysing Reagent – Prepared according to CT-BW-REAG-SOP-3, Preparation of Whole Blood Lysing Reagent
- 1% Paraformaldehyde Solution – Prepared according to CT-BW-REAG-SOP-4, Preparation of 1% Paraformaldehyde Solution

DATA ANALYSIS

After acquisition, the listmode files were analyzed offline using WinList [Verity Software] 7.0 to generate the reportables described above. Gating scheme is located in Appendix CC.

ABBREVIATIONS

The following abbreviations may appear in the text of this report or in the attached tables.

- tSTAT3 – Signal Transducer and Activator of Transcription 3, Total protein
- pSTAT3 – Signal Transducer and Activator of Transcription 3, phosphorylated at Tyrosine residue 705
- Y – Tyrosine
- %CV -- Coefficient of Variation = 100 x [Standard Deviation/Mean]
- DI – Deionized
- ELISA – Enzyme-Linked Immunosorbent Assay
- QC – Quality Control
- RT – Room Temperature (20 to 25°C)
- Universal Precautions -- Treating all patient samples as though they are infectious.
- FMO – Fluorescence Minus One
- CD – Cluster of Differentiation
- PBS-Phosphate buffered saline
- BSA – Bovine Serum Albumin
- WBC – White Blood Cell
- RBC – Red Blood Cell
- FITC - Fluorescein Isothiocyanate
- PE - Phycoerythrin
- µL – Microliter
- mL – Milliliter
- BD – Becton Dickinson
- QC – Quality Control
- LCT – LabCorp Clinical Trials
- RCF – Relative Centrifugal Force
- FACSCanto II – Fluorescence Activated Cell Sorter Canto II
- SOP – Standard Operating Procedure

REFERENCES

- CT-BW-SS-SOP-398, Gilead Specific Flow Cytometry Assay to Detect STAT3 in IL6 Stimulated Whole
- CT-BW-MAINT-SOP-30, Daily Startup, Shutdown, and Maintenance of the Becton Dickinson FACSCanto II Flow Cytometer
- CT-BW-MAINT-SOP-33, Daily Quality Control of the Becton Dickinson FACSCanto II (RUO) Flow Cytometer
- CT-BW-REAG-SOP-14, Preparation of 1X Phosphate-Buffered Saline with 2% Bovine Albumin (PBS-BSA with azide), 1X Phosphate-Buffered Saline with 1% Serum Bovine Albumin (PBS with 1% BSA with azide), and 0.5% Serum bovine Albumin (PBS-BSA without azide)
- CT-BW-REAG-SOP-3, Preparation of Whole Blood Lysing Reagent
- CT-BW-REAG-SOP-4, Preparation of 1% Paraformaldehyde Solution
- LA-LCT Assay Qualification Plan GEN82, Cross Laboratory Qualification of Human STAT3 [pY705] ELISA (cell lysate, units/mL)
- White paper: O'Hara et al. 2011. Recommendations for the validation of flow cytometric testing during drug development: II assays. Journal of Immunological Methods. 363 120-134

ACCEPTANCE CRITERIA

Linearity Acceptance Criteria

The linearity was calculated by plotting the expected values at 2000 cells by the measured values a other cell densities. The relative error was then calculated between the values using the equation below:
Equation 2: ABSOLUTE VALUE of the following: %Relative Error (%RE) = [Accepted Value (@2000 cells) – Measured Value (@N-spike])/ Accepted Value (@2000 Cells)*100.

Acceptance failed when the % Relative Error was greater than 25%. %CVs were also calculated between replicates in this phase. Acceptable %CVs for replicates was less than or equal to 20%

Inter-Operator Precision Acceptance Criteria

%CVs was calculated between operators. %CV should be ≤25%, with the possible exception of those reportables derived from populations that are present at low frequencies (<5% of the primary population, i.e. lymphocytes). For these discrete populations, the %CV may be higher than 25%, but may not reflect any trend that would indicate that the ability to detect these populations is significantly affected by stability or donor variances between collections.

Intra-Assay Precision Acceptance Criteria

%CVs was calculated within the replicates for Intra-Assay Precision. %CV should be ≤25% within donor replicates, with the possible exception of those reportables derived from populations that are present at low frequencies (<5% of the primary population, i.e. lymphocytes). For these discrete populations, the %CV may be higher than 25%, but may not reflect any trend that would indicate that the ability to detect these populations is significantly affected by stability or normal donor variances between collections.

Assay Transfer

%CVs should be the same as Intra-Assay Precision. Additionally, the percentage of stimulation of pSTAT3 should be reflective of what has been seen in normal donors in the course of the validation in Phases I-III. Inter-Donor variability will be determined from data in Phase I-III. The stimulation percentage from Assay Transfer should be within this variability plus/minus 1 standard deviation.

RESULTS

Phase I: Antibody Titration

This phase was conducted in order to titrate all reagents to saturation within the client method. Data for this phase is routinely held at LCT Brentwood facility. Titration results only are supplied as part of the validation report. The pSTAT3-PE was used at manufacturer recommended volume of 20µL/test. The tSTAT3-PE was used at manufacturer recommended volume of 20µL/test. The CD3-FITC was used at manufacturer recommended volume of 20µL/test. The CD4-V450 was used at manufacturer recommended volume of 5µL/test.

Phase II: Assay Linearity

In order to determine the minimum number of lymphocytes required to accurately determine the MESF/MFI, the 2000 event sample was used as the basis for determining the relative error of the MESF/MFI and the % positivity. Data for Linearity experiments are located in Appendices A-R. %CVs that are greater than 20% are flagged in red font throughout the tables. The CVs for pSTAT3 percentages, MFIs and MESFs were consistently acceptable in Donors 2 and 3 for all region counts of greater than 100 events. Donor 1 demonstrated increased variability in the 300 event sample due to an outlier in replicate 2. The calculated %RE reflected these same results, with the acceptance criteria of less than 25% RE being met when greater than 100 events were acquired for Donors 2 and 3, and greater than 300 events for Donor 1. All data outside acceptance criteria are flagged in red within the data tables. For the course of the study, the window of acquisition was set such that greater than 300 events must be acquired (actual stop gate was set to 10,000 lymphocytes).

Phase III: Inter-Operator and Intra-Assay Precision

A minimum of 2 normal whole blood specimens were drawn into NaHeparin. The samples were shipped to back to the Brentwood LCT within 1 Day ex-vivo using the Credo GTC4L, 2-8°C shippers from World Courier. The samples were tested immediately within the panel by 2 operators in triplicate. The acquisition stop gate was set to acquire 2000 lymphocytes per client request in this phase. Data for this phase are located in Appendices S-W. %CVs outside of the acceptance range occurred for inter-operator only for the %pSTAT3 unstimulated in the CD3+ lymphs and CD3+/CD4+ lymphs, though MESF values for these same populations were acceptable. These values are marked in red. All other values were within acceptance criteria.

Phase IV: Assay Transfer -- ELISA testing only

Reference LA-LCT Assay Qualification Plan GEN82, Cross Laboratory Qualification of Human STAT3 [pY705] ELISA (cell lysate, units/mL). Data are not included in this report.

Phase IV: Assay Transfer – Flow Cytometry testing only

The Brentwood facility drew 3 NWB donors and ship to LCT Cranford flow lab using World Courier Credo GCT4L, 2-8°C shippers. The samples were processed according to CT-BW-SS-SOP-398, Gilead Specific Flow Cytometry Assay to Detect STAT3 in IL6 Stimulated Whole Blood, in triplicate by one operator. Mean, Standard deviation and %CVs were calculated between replicates. Data for the replicate statistics and raw data are located in Appendices X-BB. Values outside acceptance criteria are marked in red font. There was one outlier in the percentage of both total and phospho STAT3 in the CD3 and CD3+/CD4+ populations for Donor 2A and Donor 3A samples. The other 2 replicates were within acceptable ranges. The primary parameters of interest are the MFI and the MESF measurements, so the assay transfer is acceptable, as these are within acceptance guidelines. In addition, in Appendix DD, the percentage of stimulation was calculated for each normal donor tested in all validation phases. The mean and standard deviation, then mean + 1 standard deviation and mean -1 standard deviation were calculated. All percentages of stimulations that did

not fall within the Mean +/- 1StDev were flagged in pink highlight with red font. All AT samples were within acceptance criteria. Precision Donor 2 and Linearity Donor 1 were outside the 1 standard deviation in the % stimulation based on the MESF in CD3+ (Lymphs). These values were lower in these 2 donors than any other. This is considered normal for these donors.

Conclusion

These experiments demonstrate that the Gilead pSTAT3 Stimulation Assay is performed with precision. Specimens were received and analyzed up to Day 2 (48-54 hours, per client internal data, not included) when collected in sodium heparin vacutainers and shipped/stored at 2-8°C. The assay transfer experiments to the Cranford Laboratory demonstrated equivalent stimulation and biological variability as seen within the validation studies.

Appendix A: Table 1, Linearity – Healthy Donors 1-3

Linearity Table 1	Acquisition Date	%Lymphs	Region Events CD3+ Lymphs	%CD3+ [Lymphs]	Region Events CD3+/CD4+ Lymphs	%CD3+/CD4 [Lymphs]	MESF FMO-PE unstim [CD3+ Lymphs]	MESF FMO-PE unstim [CD3+/CD4+ Lymphs]	%tSTAT3 unstim [CD3+ Lymphs]
D1-2000-R1	2/5/2014	34.1	1369	77.8	608	44.4	533	533	41.4
D1-2000-R2	2/5/2014	31.4	1382	78.7	581	42.0	515	459	37.5
D1-2000-R3	2/5/2014	33.9	1437	79.4	629	43.8	540	540	37.1
Mean		33.1	1396	78.6	606	43.4	529	511	38.7
StDev		1.5	36.1	0.8	24.1	1.2	12.9	44.9	2.4
%CV		4.5	2.6	1.0	4.0	2.8	2.4	8.8	6.2
D1-1000-R1	2/5/2014	33.0	686	76.9	261	38.1	548	540	38.3
D1-1000-R2	2/5/2014	32.6	690	77.6	299	43.3	533	540	31.2
D1-1000-R3	2/5/2014	32.2	683	78.3	297	43.5	536	504	36.6
Mean		32.6	686	77.6	286	41.6	539	528	35.4
StDev		0.4	3.5	0.7	21.4	3.1	7.9	20.8	3.7
%CV		1.2	0.5	0.9	7.5	7.5	1.5	3.9	10.5
D1-500-R1	2/5/2014	31.8	354	79.2	173	48.9	524	507	31.7
D1-500-R2	2/5/2014	32.9	347	77.5	127	36.6	571	557	30.3
D1-500-R3	2/5/2014	33.7	358	78.5	151	42.2	588	597	27.3
Mean		32.8	353	78.4	150	42.6	561	554	29.8
StDev		1.0	5.6	0.9	23.0	6.2	33.2	45.1	2.2
%CV		3.0	1.6	1.1	15.3	14.6	5.9	8.1	7.4
D1-300-R1	2/5/2014	30.8	213	79.8	86	40.4	540	589	29.0
D1-300-R2	2/5/2014	35.9	199	71.8	89	44.7	531	586	24.6
D1-300-R3	2/5/2014	32.0	221	80.7	100	45.3	570	588	21.6
Mean		32.9	211	77.4	92	43.5	547	588	25.1
StDev		2.7	11.1	4.9	7.4	2.7	20.4	1.5	3.7
%CV		8.2	5.3	6.3	8.0	6.2	3.7	0.3	14.7
D1-100-R1	2/5/2014	34.6	71	84.5	36	50.7	582	537	19.4
D1-100-R2	2/5/2014	32.0	71	76.3	26	36.6	575	647	23.7
D1-100-R3	2/5/2014	32.3	65	75.6	21	32.3	547	504	13.0
Mean		33.0	69	78.8	28	39.9	568	563	18.7
StDev		1.4	3.5	4.9	7.6	9.6	18.5	74.9	5.4
%CV		4.2	5.1	6.2	27.1	24.1	3.3	13.3	28.9
D1-50-R1	2/5/2014	31.9	38	86.4	15	39.5	507	413	32.4
D1-50-R2	2/5/2014	24.6	37	84.1	22	59.5	501	771	9.1
D1-50-R3	2/5/2014	32.4	30	66.7	11	36.7	519	655	14.3
Mean		29.6	35	79.1	16	45.2	509	613	18.6
StDev		4.4	4.4	10.8	5.6	12.4	9.2	182.7	12.2
%CV		14.9	12.6	13.7	35.0	27.4	1.8	29.8	65.6

Appendix B: Table 1, Linearity – Healthy Donors 1-3 – Continued

Linearity Table 1 Continued	Acquisition Date	%tSTAT3 unstim [CD3+/CD4+ Lymphs]	MESF tSTAT3 unstim [CD3+ Lymphs]	MESF tSTAT3 unstim [CD3+/CD4+ Lymphs]	%pSTAT3 unstim [CD3+ Lymphs]	%pSTAT3 unstim [CD3+/CD4+ Lymphs]	MESF pSTAT3 unstim [CD3+ Lymphs]	MESF pSTAT3 unstim [CD3+/CD4+ Lymphs]	MESF FMO-PE STIM [CD3+ Lymphs]	MESF FMO-PE STIM [CD3+/CD4+ Lymphs]
D1-2000-R1	2/5/2014	54.4	1669	1686	2.6	6.4	739	777	520	506
D1-2000-R2	2/5/2014	48.0	1618	1607	2.8	6.3	734	790	520	532
D1-2000-R3	2/5/2014	49.0	1611	1609	1.8	4.9	733	815	538	533
Mean		50.5	1633	1634	2.4	5.9	735	794	526	524
StDev		3.4	31.7	45.0	0.5	0.8	3.2	19.3	10.4	15.3
%CV		6.7	1.9	2.8	20.8	13.6	0.4	2.4	2.0	2.9
D1-1000-R1	2/5/2014	44.9	1607	1581	3.1	6.6	746	800	555	569
D1-1000-R2	2/5/2014	42.7	1563	1557	3.1	7.5	751	837	542	504
D1-1000-R3	2/5/2014	44.4	1562	1543	1.9	5.7	757	818	538	517
Mean		44.0	1577	1560	2.7	6.6	751	818	545	530
StDev		1.2	25.7	19.2	0.7	0.9	5.5	18.5	8.9	34.4
%CV		2.7	1.6	1.2	25.9	13.6	0.7	2.3	1.6	6.5
D1-500-R1	2/5/2014	44.7	1504	1537	2.3	7.0	713	805	557	580
D1-500-R2	2/5/2014	36.0	1479	1456	3.2	6.2	756	813	571	570
D1-500-R3	2/5/2014	37.3	1523	1429	2.0	5.8	743	820	565	549
Mean		39.3	1502	1474	2.5	6.3	737	813	564	566
StDev		4.7	22.1	56.2	0.6	0.6	22.1	7.5	7.0	15.8
%CV		12.0	1.5	3.8	24.0	9.5	3.0	0.9	1.2	2.8
D1-300-R1	2/5/2014	44.4	1463	1451	0.9	2.0	754	754	577	582
D1-300-R2	2/5/2014	37.4	1488	1547	2.4	4.8	747	805	580	589
D1-300-R3	2/5/2014	32.4	1429	1403	1.4	8.1	753	815	560	501
Mean		38.1	1460	1467	1.6	5.0	751	791	572	557
StDev		6.0	29.6	73.3	0.8	3.1	3.8	32.7	10.8	48.9
%CV		15.7	2.0	5.0	50.0	62.0	0.5	4.1	1.9	8.8
D1-100-R1	2/5/2014	26.9	1398	1343	2.9	6.1	735	790	557	509
D1-100-R2	2/5/2014	38.5	1351	1411	1.5	3.5	667	761	440	343
D1-100-R3	2/5/2014	21.9	1414	1420	0.0	5.0	724	841	582	495
Mean		29.1	1388	1391	1.5	4.9	709	797	526	449
StDev		8.5	32.7	42.1	1.5	1.3	36.5	40.5	75.8	92.1
%CV		29.2	2.4	3.0	100.0	26.8	5.1	5.1	14.4	20.5
D1-50-R1	2/5/2014	37.5	1447	1484	0.0	4.8	676	672	808	582
D1-50-R2	2/5/2014	41.2	1420	1550	2.9	5.9	788	771	512	478
D1-50-R3	2/5/2014	30.8	1178	1351	3.1	5.6	825	857	631	582
Mean		36.5	1348	1462	2.0	5.4	763	767	650	547
StDev		5.3	148.1	101.4	1.7	0.6	77.6	92.6	148.9	60.0
%CV		14.5	11.0	6.9	85.0	11.1	10.2	12.1	22.9	11.0

Appendix C: Table 1, Linearity – Healthy Donors 1-3 – Continued

Linearity Table 1 Continued	Acquisition Date	%tSTAT3 STIM [CD3+ Lymphs]	%tSTAT3 STIM [CD3+/CD4+ Lymphs]	MESF tSTAT3 STIM [CD3+ Lymphs]	MESF tSTAT3 STIM [CD3+/CD4+ Lymphs]	%pSTAT3 STIM [CD3+ Lymphs]	%pSTAT3 STIM [CD3+/CD4+ Lymphs]	MESF pSTAT3 STIM [CD3+ Lymphs]	MESF pSTAT3 STIM [CD3+/CD4+ Lymphs]
D1-2000-R1	2/5/2014	38.4	47.4	1625	1597	54.7	93.8	3063	10779
D1-2000-R2	2/5/2014	36.2	46.9	1610	1585	53.3	92.8	2365	10704
D1-2000-R3	2/5/2014	35.3	45.4	1584	1566	54.7	93.6	3039	10463
Mean		36.6	46.6	1606	1583	54.2	93.4	2822	10649
StDev		1.6	1.0	20.7	15.6	0.8	0.5	396.2	165.1
%CV		4.4	2.1	1.3	1.0	1.5	0.5	14.0	1.6
D1-1000-R1	2/5/2014	34.7	44.4	1551	1534	53.2	95.4	2600	10568
D1-1000-R2	2/5/2014	28.9	39.4	1500	1487	55.4	93.7	2885	10367
D1-1000-R3	2/5/2014	32.4	39.8	1562	1547	55.1	95.3	2882	10159
Mean		32.0	41.2	1538	1523	54.6	94.8	2789	10365
StDev		2.9	2.8	33.1	31.6	1.2	1.0	163.7	204.5
%CV		9.1	6.8	2.2	2.1	2.2	1.1	5.9	2.0
D1-500-R1	2/5/2014	28.4	35.3	1512	1461	57.6	93.6	4558	9971
D1-500-R2	2/5/2014	30.1	35.8	1525	1400	47.8	92.1	1456	10096
D1-500-R3	2/5/2014	22.9	25.9	1420	1381	53.9	91.4	2891	9924
Mean		27.1	32.3	1486	1414	53.1	92.4	2968	9997
StDev		3.8	5.6	57.2	41.8	4.9	1.1	1552.4	88.9
%CV		14.0	17.3	3.8	3.0	9.2	1.2	52.3	0.9
D1-300-R1	2/5/2014	32.4	38.0	1527	1490	49.3	95.4	1670	10646
D1-300-R2	2/5/2014	21.8	30.4	1405	1405	54.8	92.1	3116	9368
D1-300-R3	2/5/2014	22.8	29.6	1420	1405	58.8	94.0	3665	9555
Mean		25.7	32.7	1451	1433	54.3	93.8	2817	9856
StDev		5.9	4.6	66.5	49.1	4.8	1.7	1030.6	690.2
%CV		23.0	14.1	4.6	3.4	8.8	1.8	36.6	7.0
D1-100-R1	2/5/2014	26.9	31.8	1507	1530	54.9	97.2	5196	8829
D1-100-R2	2/5/2014	18.6	21.7	1272	1254	46.5	88.5	1589	9281
D1-100-R3	2/5/2014	28.4	39.4	1377	1407	43.1	90.5	1285	8347
Mean		24.6	31.0	1385	1397	48.2	92.1	2690	8819
StDev		5.3	8.9	117.7	138.3	6.1	4.6	2175.6	467.1
%CV		21.5	28.7	8.5	9.9	12.7	5.0	80.9	5.3
D1-50-R1	2/5/2014	13.8	25.0	1193	1156	68.4	100.0	6627	11689
D1-50-R2	2/5/2014	10.5	40.0	1277	1550	67.6	95.5	5005	8720
D1-50-R3	2/5/2014	20.0	27.8	1407	1215	43.3	90.9	1474	7368
Mean		14.8	30.9	1292	1307	59.8	95.5	4369	9259
StDev		4.8	8.0	107.8	212.5	14.3	4.6	2634.8	2210.4
%CV		32.4	25.9	8.3	16.3	23.9	4.8	60.3	23.9

Appendix D: Table 1, Linearity – Healthy Donors 1-3 – Continued

Linearity Table 1 Continued	Acquisition Date	MFI FMO-PE unstim [CD3+ Lymphs]	MFI FMO-PE unstim [CD3+/CD4+ Lymphs]	MFI tSTAT3 unstim [CD3+ Lymphs]	MFI tSTAT3 unstim [CD3+/CD4+ Lymphs]	MFI pSTAT3 unstim [CD3+ Lymphs]	MFI pSTAT3 unstim [CD3+/CD4+ Lymphs]
D1-2000-R1	2/5/2014	218	218	665	671	300	315
D1-2000-R2	2/5/2014	211	188	645	641	298	320
D1-2000-R3	2/5/2014	221	221	642	641	298	330
Mean		217	209	651	651	299	322
StDev		5.1	18.2	12.5	17.3	1.2	7.6
%CV		2.4	8.7	1.9	2.7	0.4	2.4
D1-1000-R1	2/5/2014	224	221	641	631	303	324
D1-1000-R2	2/5/2014	218	221	623	621	305	339
D1-1000-R3	2/5/2014	219	207	623	616	307	331
Mean		220	216	629	623	305	331
StDev		3.2	8.1	10.4	7.6	2.0	7.5
%CV		1.5	3.8	1.7	1.2	0.7	2.3
D1-500-R1	2/5/2014	214	208	601	613	290	326
D1-500-R2	2/5/2014	233	228	591	582	307	329
D1-500-R3	2/5/2014	240	243	608	571	302	332
Mean		229	226	600	589	300	329
StDev		13.5	17.6	8.5	21.8	8.7	3.0
%CV		5.9	7.8	1.4	3.7	2.9	0.9
D1-300-R1	2/5/2014	221	241	585	580	306	306
D1-300-R2	2/5/2014	217	239	594	617	303	326
D1-300-R3	2/5/2014	233	240	571	561	306	330
Mean		224	240	583	586	305	321
StDev		8.3	1.0	11.6	28.5	1.7	12.9
%CV		3.7	0.4	2.0	4.9	0.6	4.0
D1-100-R1	2/5/2014	238	220	559	538	298	320
D1-100-R2	2/5/2014	235	264	541	564	271	309
D1-100-R3	2/5/2014	224	207	566	568	294	340
Mean		232	230	555	557	288	323
StDev		7.4	29.9	12.9	16.3	14.6	15.7
%CV		3.2	13.0	2.3	2.9	5.1	4.9
D1-50-R1	2/5/2014	208	170	578	593	275	273
D1-50-R2	2/5/2014	205	313	568	618	319	313
D1-50-R3	2/5/2014	212	267	473	541	334	347
Mean		208	250	540	584	309	311
StDev		3.5	73.0	58.0	39.3	30.7	37.0
%CV		1.7	29.2	10.7	6.7	9.9	11.9

Appendix E: Table 1, Linearity – Healthy Donors 1-3 – Continued

Linearity Table 1 Continued	Acquisition Date	MFI FMO-PE STIM [CD3+ Lymphs]	MFI FMO-PE STIM [CD3+/CD4+ Lymphs]	MFI tSTAT3 STIM [CD3+ Lymphs]	MFI tSTAT3 STIM [CD3+/CD4+ Lymphs]	MFI pSTAT3 STIM [CD3+ Lymphs]	MFI pSTAT3 STIM [CD3+/CD4+ Lymphs]
D1-2000-R1	2/5/2014	213	207	648	637	1203	4113
D1-2000-R2	2/5/2014	213	218	642	632	935	4085
D1-2000-R3	2/5/2014	220	218	632	625	1194	3995
Mean		215	214	641	631	1111	4064
StDev		4.0	6.4	8.1	6.0	152.2	61.7
%CV		1.9	3.0	1.3	1.0	13.7	1.5
D1-1000-R1	2/5/2014	227	232	619	612	1025	4034
D1-1000-R2	2/5/2014	222	207	599	594	1135	3959
D1-1000-R3	2/5/2014	220	212	623	617	1134	3881
Mean		223	217	614	608	1098	3958
StDev		3.6	13.2	12.9	12.1	63.2	76.5
%CV		1.6	6.1	2.1	2.0	5.8	1.9
D1-500-R1	2/5/2014	228	237	604	584	1774	3811
D1-500-R2	2/5/2014	233	233	609	560	582	3858
D1-500-R3	2/5/2014	231	225	568	553	1137	3794
Mean		231	232	594	566	1164	3821
StDev		2.5	6.1	22.4	16.3	596.5	33.2
%CV		1.1	2.6	3.8	2.9	51.2	0.9
D1-300-R1	2/5/2014	235	238	610	595	665	4063
D1-300-R2	2/5/2014	237	241	562	562	1224	3586
D1-300-R3	2/5/2014	229	205	568	562	1434	3656
Mean		234	228	580	573	1108	3768
StDev		4.2	20.0	26.2	19.1	397.5	257.6
%CV		1.8	8.8	4.5	3.3	35.9	6.8
D1-100-R1	2/5/2014	228	208	602	611	2016	3384
D1-100-R2	2/5/2014	181	142	510	503	634	3553
D1-100-R3	2/5/2014	238	203	551	563	515	3204
Mean		216	184	554	559	1055	3380
StDev		30.4	36.7	46.1	54.1	834.4	174.5
%CV		14.1	19.9	8.3	9.7	79.1	5.2
D1-50-R1	2/5/2014	327	238	479	464	2557	4452
D1-50-R2	2/5/2014	210	196	512	618	1944	3343
D1-50-R3	2/5/2014	257	238	563	488	563	488
Mean		265	224	518	523	1688	2761
StDev		58.9	24.2	42.3	82.9	1021.4	2045.1
%CV		22.2	10.8	8.2	15.9	60.5	74.1

Appendix F: Table 1, Linearity – Healthy Donors 1-3 – Continued

Table 1: Linearity	Acquisition Date	%Lymphs	Region Events CD3+ Lymphs	%CD3+ [Lymphs]	Region Events CD3+/CD4+ Lymphs	%CD3+/CD4 [Lymphs]	MESF FMO-PE unstim [CD3+ Lymphs]	MESF FMO-PE unstim [CD3+/CD4+ Lymphs]	%STAT3 unstim [CD3+ Lymphs]
D2-2000-R1	2/5/2014	18.8	1427	72.7	1055	73.9	418	414	88.6
D2-2000-R2	2/5/2014	18.6	1391	71.5	1027	73.8	430	425	84.7
D2-2000-R3	2/5/2014	19.1	1416	72.8	1031	72.8	412	409	85.2
Mean		18.8	1411	72.3	1038	73.5	420	416	86.2
StDev		0.3	18.4	0.7	15.1	0.6	9.2	8.2	2.1
%CV		1.6	1.3	1.0	1.5	0.8	2.2	2.0	2.4
D2-1000-R1	2/5/2014	18.2	684	70.6	492	71.9	385	380	85.0
D2-1000-R2	2/5/2014	19.0	709	72.3	515	72.6	428	432	81.5
D2-1000-R3	2/5/2014	18.7	715	73.5	525	73.4	432	413	82.9
Mean		18.6	703	72.1	511	72.6	415	408	83.1
StDev		0.4	16.4	1.5	16.9	0.8	26.1	26.3	1.8
%CV		2.2	2.3	2.1	3.3	1.1	6.3	6.4	2.2
D2-500-R1	2/5/2014	18.4	336	68.7	258	76.8	398	393	78.8
D2-500-R2	2/5/2014	17.8	323	68.0	246	76.2	398	383	64.4
D2-500-R3	2/5/2014	18.8	350	71.4	262	74.9	477	457	64.1
Mean		18.3	336	69.4	255	76.0	424	411	69.1
StDev		0.5	13.5	1.8	8.3	1.0	45.6	40.1	8.4
%CV		2.7	4.0	2.6	3.3	1.3	10.8	9.8	12.2
D2-300-R1	2/5/2014	17.8	211	72.3	154	73.0	468	477	63.5
D2-300-R2	2/5/2014	21.5	217	71.9	157	72.4	474	526	64.0
D2-300-R3	2/5/2014	18.0	210	71.7	160	76.2	469	467	54.0
Mean		19.1	213	72.0	157	73.9	470	490	60.5
StDev		2.1	3.8	0.3	3.0	2.0	3.2	31.6	5.6
%CV		11.0	1.8	0.4	1.9	2.7	0.7	6.4	9.3
D2-100-R1	2/5/2014	17.4	71	70.3	59	83.1	564	655	53.7
D2-100-R2	2/5/2014	22.6	67	69.8	49	73.1	426	403	52.4
D2-100-R3	2/5/2014	19.1	69	70.4	50	72.5	481	480	51.4
Mean		19.7	69	70.2	53	76.2	490	513	52.5
StDev		2.7	2.0	0.3	5.5	6.0	69.5	129.1	1.2
%CV		13.7	2.9	0.4	10.4	7.9	14.2	25.2	2.3
D2-50-R1	2/5/2014	20.3	33	68.8	26	78.8	627	465	61.9
D2-50-R2	2/5/2014	18.6	33	67.4	25	75.8	480	501	42.4
D2-50-R3	2/5/2014	22.7	41	83.7	28	68.3	480	501	45.2
Mean		20.5	36	73.3	26	74.3	529	489	49.8
StDev		2.1	4.6	9.0	1.5	5.4	84.9	20.8	10.5
%CV		10.2	12.8	12.3	5.8	7.3	16.0	4.3	21.1

Appendix G: Table 1, Linearity – Healthy Donors 1-3 – Continued

Table 1: Linearity - Continued	Acquisition Date	%tSTAT3 unstim [CD3+/CD4+ Lymphs]	MESF tSTAT3 unstim [CD3+ Lymphs]	MESF tSTAT3 unstim [CD3+/CD4+ Lymphs]	%pSTAT3 unstim [CD3+ Lymphs]	%pSTAT3 unstim [CD3+/CD4+ Lymphs]	MESF pSTAT3 unstim [CD3+ Lymphs]	MESF pSTAT3 unstim [CD3+/CD4+ Lymphs]	MESF FMO-PE STIM [CD3+ Lymphs]	MESF FMO-PE STIM [CD3+/CD4+ Lymphs]
D2-2000-R1	2/5/2014	83.3	1852	1848	50.1	51.9	1238	1407	434	423
D2-2000-R2	2/5/2014	79.4	1761	1758	50.7	51.1	1245	1386	451	452
D2-2000-R3	2/5/2014	79.0	1779	1764	48.5	52.0	1202	1405	454	435
Mean		80.6	1797	1790	49.8	51.7	1228	1399	446	437
StDev		2.4	48.2	50.3	1.1	0.5	23.1	11.6	10.8	14.6
%CV		3.0	2.7	2.8	2.2	1.0	1.9	0.8	2.4	3.3
D2-1000-R1	2/5/2014	79.7	1707	1702	44.3	47.4	1137	1301	454	449
D2-1000-R2	2/5/2014	76.2	1692	1688	48.5	52.2	1204	1390	450	435
D2-1000-R3	2/5/2014	75.6	1642	1662	49.7	50.5	1225	1368	442	438
Mean		77.2	1680	1684	47.5	50.0	1189	1353	449	441
StDev		2.2	34.0	20.3	2.8	2.4	46.0	46.4	6.1	7.4
%CV		2.8	2.0	1.2	5.9	4.8	3.9	3.4	1.4	1.7
D2-500-R1	2/5/2014	70.8	1587	1599	47.9	49.6	1194	1340	383	398
D2-500-R2	2/5/2014	53.7	1415	1422	52.7	53.1	1296	1447	440	418
D2-500-R3	2/5/2014	52.0	1400	1403	46.8	48.6	1178	1332	498	481
Mean		58.8	1467	1475	49.1	50.4	1223	1373	440	432
StDev		10.4	103.9	108.1	3.1	2.4	64.0	64.2	57.5	43.3
%CV		17.7	7.1	7.3	6.3	4.8	5.2	4.7	13.1	10.0
D2-300-R1	2/5/2014	52.8	1389	1408	47.0	52.4	1161	1432	511	511
D2-300-R2	2/5/2014	51.7	1418	1389	49.5	52.1	1225	1429	492	480
D2-300-R3	2/5/2014	46.0	1269	1323	54.0	53.5	1318	1433	451	437
Mean		50.2	1359	1373	50.2	52.7	1235	1431	485	476
StDev		3.7	79.0	44.6	3.5	0.7	78.9	2.1	30.7	37.2
%CV		7.4	5.8	3.2	7.0	1.3	6.4	0.1	6.3	7.8
D2-100-R1	2/5/2014	51.9	1301	1385	41.3	44.2	1088	1163	471	474
D2-100-R2	2/5/2014	39.1	1254	1254	53.6	53.3	1281	1385	524	527
D2-100-R3	2/5/2014	44.2	1277	1330	40.9	39.2	1066	1120	409	424
Mean		45.1	1277	1323	45.3	45.6	1145	1223	468	475
StDev		6.4	23.5	65.8	7.2	7.1	118.3	142.2	57.6	51.5
%CV		14.2	1.8	5.0	15.9	15.6	10.3	11.6	12.3	10.8
D2-50-R1	2/5/2014	56.3	1301	1411	36.4	43.5	1130	1139	575	575
D2-50-R2	2/5/2014	33.3	1193	1230	45.2	40.9	1208	1215	554	471
D2-50-R3	2/5/2014	33.3	1223	1167	36.1	45.8	995	1170	517	416
Mean		41.0	1239	1269	39.2	43.4	1111	1175	549	487
StDev		13.3	55.7	126.7	5.2	2.5	107.8	38.2	29.4	80.7
%CV		32.4	4.5	10.0	13.3	5.8	9.7	3.3	5.4	16.6

Appendix H: Table 1, Linearity – Healthy Donors 1-3 – Continued

Table 1: Linearity - Continued	Acquisition Date	%tSTAT3 STIM [CD3+ Lymphs]	%tSTAT3 STIM [CD3+/CD4+ Lymphs]	MESF tSTAT3 STIM [CD3+ Lymphs]	MESF tSTAT3 STIM [CD3+/CD4+ Lymphs]	%pSTAT3 STIM [CD3+ Lymphs]	%pSTAT3 STIM [CD3+/CD4+ Lymphs]	MESF pSTAT3 STIM [CD3+ Lymphs]	MESF pSTAT3 STIM [CD3+/CD4+ Lymphs]
D2-2000-R1	2/5/2014	82.4	72.5	1679	1672	91.0	99.4	13130	13881
D2-2000-R2	2/5/2014	80.4	72.8	1683	1678	89.9	99.1	12904	13713
D2-2000-R3	2/5/2014	81.1	72.8	1685	1672	89.5	99.1	13103	13897
Mean		81.3	72.7	1682	1674	90.1	99.2	13046	13830
StDev		1.0	0.2	3.1	3.5	0.8	0.2	123.4	101.9
%CV		1.2	0.3	0.2	0.2	0.9	0.2	0.9	0.7
D2-1000-R1	2/5/2014	77.3	66.4	1590	1547	87.7	98.6	12645	13643
D2-1000-R2	2/5/2014	76.3	65.7	1574	1570	89.1	98.6	12588	13330
D2-1000-R3	2/5/2014	76.2	66.5	1579	1568	91.8	98.7	12568	13242
Mean		76.6	66.2	1581	1562	89.5	98.6	12600	13405
StDev		0.6	0.4	8.2	12.7	2.1	0.1	40.0	210.8
%CV		0.8	0.6	0.5	0.8	2.3	0.1	0.3	1.6
D2-500-R1	2/5/2014	77.8	65.5	1565	1563	88.4	98.8	11960	12932
D2-500-R2	2/5/2014	68.6	59.8	1472	1469	91.3	99.6	12079	12916
D2-500-R3	2/5/2014	65.6	58.4	1442	1464	90.9	99.2	11617	12403
Mean		70.7	61.2	1493	1499	90.2	99.2	11885	12750
StDev		6.4	3.8	64.1	55.8	1.6	0.4	239.9	300.9
%CV		9.1	6.2	4.3	3.7	1.8	0.4	2.0	2.4
D2-300-R1	2/5/2014	68.7	60.6	1477	1484	87.7	96.8	11419	11836
D2-300-R2	2/5/2014	64.2	52.5	1363	1370	90.8	96.8	10949	11836
D2-300-R3	2/5/2014	61.1	51.9	1377	1372	90.0	98.8	11401	12262
Mean		64.7	55.0	1406	1409	89.5	97.5	11256	11978
StDev		3.8	4.9	62.2	65.2	1.6	1.2	266.3	246.0
%CV		5.9	8.9	4.4	4.6	1.8	1.2	2.4	2.1
D2-100-R1	2/5/2014	62.8	60.4	1394	1456	88.7	98.3	9847	10983
D2-100-R2	2/5/2014	68.2	59.5	1370	1377	92.5	100.0	11985	12085
D2-100-R3	2/5/2014	58.6	41.9	1310	1269	89.9	100.0	11689	12520
Mean		63.2	53.9	1358	1367	90.4	99.4	11174	11863
StDev		4.8	10.4	43.3	93.9	1.9	1.0	1158.4	792.3
%CV		7.6	19.3	3.2	6.9	2.1	1.0	10.4	6.7
D2-50-R1	2/5/2014	53.1	37.5	1277	1142	90.9	96.2	11641	11714
D2-50-R2	2/5/2014	59.0	37.0	1334	1273	84.9	100.0	11689	12442
D2-50-R3	2/5/2014	40.5	34.4	1142	1079	95.1	100.0	11086	10914
Mean		50.9	36.3	1251	1165	90.3	98.7	11472	11690
StDev		9.5	1.7	98.6	99.0	5.1	2.2	335.1	764.3
%CV		18.7	4.7	7.9	8.5	5.6	2.2	2.9	6.5

Appendix I: Table 1, Linearity – Healthy Donors 1-3 – Continued

Table 1: Linearity Continued	Acquisition Date	MFI FMO-PE unstim [CD3+ Lymphs]	MFI FMO-PE unstim [CD3+/CD4+ Lymphs]	MFI tSTAT3 unstim [CD3+ Lymphs]	MFI tSTAT3 unstim [CD3+/CD4+ Lymphs]	MFI pSTAT3 unstim [CD3+ Lymphs]	MFI pSTAT3 unstim [CD3+/CD4+ Lymphs]
D2-2000-R1	2/5/2014	172	170	736	734	497	563
D2-2000-R2	2/5/2014	177	175	701	700	499	555
D2-2000-R3	2/5/2014	170	168	708	702	483	562
Mean		173	171	715	712	493	560
StDev		3.6	3.6	18.5	19.1	8.7	4.4
%CV		2.1	2.1	2.6	2.7	1.8	0.8
D2-1000-R1	2/5/2014	159	157	680	678	457	521
D2-1000-R2	2/5/2014	176	177	674	672	483	556
D2-1000-R3	2/5/2014	178	170	654	662	492	547
Mean		171	168	669	671	477	541
StDev		10.4	10.1	13.6	8.1	18.2	18.2
%CV		6.1	6.0	2.0	1.2	3.8	3.4
D2-500-R1	2/5/2014	164	162	633	638	480	536
D2-500-R2	2/5/2014	164	158	566	569	519	578
D2-500-R3	2/5/2014	196	188	560	561	473	533
Mean		175	169	586	589	491	549
StDev		18.5	16.3	40.5	42.3	24.8	25.2
%CV		10.6	9.6	6.9	7.2	5.1	4.6
D2-300-R1	2/5/2014	192	196	556	563	467	573
D2-300-R2	2/5/2014	194	215	567	556	492	571
D2-300-R3	2/5/2014	192	192	509	530	528	573
Mean		193	201	544	550	496	572
StDev		1.2	12.3	30.8	17.4	30.7	1.2
%CV		0.6	6.1	5.7	3.2	6.2	0.2
D2-100-R1	2/5/2014	231	267	521	554	438	467
D2-100-R2	2/5/2014	175	166	503	503	514	554
D2-100-R3	2/5/2014	197	197	512	533	429	451
Mean		201	210	512	530	460	491
StDev		28.2	51.7	9.0	25.6	46.7	55.4
%CV		14.0	24.6	1.8	4.8	10.2	11.3
D2-50-R1	2/5/2014	256	191	521	564	454	458
D2-50-R2	2/5/2014	197	205	479	494	485	488
D2-50-R3	2/5/2014	197	205	491	469	401	470
Mean		217	200	497	509	447	472
StDev		34.1	8.1	21.6	49.2	42.5	15.1
%CV		15.7	4.1	4.3	9.7	9.5	3.2

Appendix J: Table 1, Linearity – Healthy Donors 1-3 – Continued

Table 1: Linearity Continued	Acquisition Date	MFI FMO-PE STIM [CD3+ Lymphs]	MFI FMO-PE STIM [CD3+/CD4+ Lymphs]	MFI tSTAT3 STIM [CD3+ Lymphs]	MFI tSTAT3 STIM [CD3+/CD4+ Lymphs]	MFI pSTAT3 STIM [CD3+ Lymphs]	MFI pSTAT3 STIM [CD3+/CD4+ Lymphs]
D2-2000-R1	2/5/2014	178	174	669	666	4987	5265
D2-2000-R2	2/5/2014	185	185	670	668	4903	5203
D2-2000-R3	2/5/2014	187	179	671	666	4977	5271
Mean		183	179	670	667	4956	5246
StDev		4.7	5.5	1.0	1.2	45.9	37.6
%CV		2.6	3.1	0.1	0.2	0.9	0.7
D2-1000-R1	2/5/2014	186	184	634	617	4807	5177
D2-1000-R2	2/5/2014	185	179	628	626	4786	5061
D2-1000-R3	2/5/2014	182	180	630	625	4778	5028
Mean		184	181	631	623	4790	5089
StDev		2.1	2.6	3.1	4.9	15.0	78.3
%CV		1.1	1.4	0.5	0.8	0.3	1.5
D2-500-R1	2/5/2014	158	164	625	623	4552	4913
D2-500-R2	2/5/2014	181	172	588	587	4596	4907
D2-500-R3	2/5/2014	204	197	577	585	4425	4717
Mean		181	178	597	598	4524	4846
StDev		23.0	17.2	25.1	21.4	88.8	111.5
%CV		12.7	9.7	4.2	3.6	2.0	2.3
D2-300-R1	2/5/2014	209	209	590	593	4351	4506
D2-300-R2	2/5/2014	202	197	545	548	4176	4506
D2-300-R3	2/5/2014	185	180	551	549	4344	4664
Mean		199	195	562	563	4290	4559
StDev		12.3	14.6	24.4	25.7	99.1	91.2
%CV		6.2	7.5	4.3	4.6	2.3	2.0
D2-100-R1	2/5/2014	193	194	558	582	3765	4189
D2-100-R2	2/5/2014	214	216	548	551	4561	4599
D2-100-R3	2/5/2014	168	174	525	509	4452	4760
Mean		192	195	544	547	4259	4516
StDev		23.0	21.0	16.9	36.6	431.6	294.4
%CV		12.0	10.8	3.1	6.7	10.1	6.5
D2-50-R1	2/5/2014	235	235	512	459	4434	4461
D2-50-R2	2/5/2014	226	193	534	510	4452	4731
D2-50-R3	2/5/2014	212	171	459	434	4227	4163
Mean		224	200	502	468	4371	4452
StDev		11.6	32.5	38.6	38.7	125.0	284.1
%CV		5.2	16.3	7.7	8.3	2.9	6.4

Appendix K: Table 1, Linearity – Healthy Donors 1-3 – Continued

Table 1 Continued	Acquisition Date	%Lymphs	Region Events CD3+ Lymphs	%CD3+ [Lymphs]	Region Events CD3+/CD4+ Lymphs	%CD3+/CD4 [Lymphs]	MESF FMO-PE unstim [CD3+ Lymphs]	MESF FMO-PE unstim [CD3+/CD4+ Lymphs]	%tSTAT3 unstim [CD3+ Lymphs]
D3-2000-R1	2/11/2014	26.6	1365	71.0	901	66.0	396	389	88.8
D3-2000-R2	2/11/2014	26.8	1371	70.6	950	69.3	414	413	88.7
D3-2000-R3	2/11/2014	27.5	1380	71.2	949	68.8	418	414	88.0
Mean		27.0	1372	70.9	933	68.0	409	405	88.5
StDev		0.5	7.5	0.3	28.0	1.8	11.7	14.2	0.4
%CV		1.9	0.5	0.4	3.0	2.6	2.9	3.5	0.5
D3-1000-R1	2/11/2014	27.3	687	70.8	482	70.2	458	439	87.4
D3-1000-R2	2/11/2014	28.0	689	70.7	463	67.2	427	432	84.9
D3-1000-R3	2/11/2014	27.4	667	69.6	458	68.7	435	416	84.5
Mean		27.6	681	70.4	468	68.7	440	429	85.6
StDev		0.4	12.2	0.7	12.7	1.5	16.1	11.8	1.6
%CV		1.4	1.8	1.0	2.7	2.2	3.7	2.8	1.9
D3-500-R1	2/11/2014	29.8	341	70.2	230	67.5	433	402	85.7
D3-500-R2	2/11/2014	28.7	336	69.9	226	67.3	428	436	82.1
D3-500-R3	2/11/2014	28.5	333	68.4	222	66.7	452	469	81.0
Mean		29.0	337	69.5	226	67.2	438	436	82.9
StDev		0.7	4.0	1.0	4.0	0.4	12.7	33.5	2.5
%CV		2.4	1.2	1.4	1.8	0.6	2.9	7.7	3.0
D3-300-R1	2/11/2014	30.4	206	71.0	139	67.5	395	378	79.6
D3-300-R2	2/11/2014	30.9	204	69.6	139	68.1	499	487	72.9
D3-300-R3	2/11/2014	31.7	195	67.5	133	68.2	451	452	74.3
Mean		31.0	202	69.4	137	67.9	448	439	75.6
StDev		0.7	5.9	1.8	3.5	0.4	52.1	55.7	3.5
%CV		2.3	2.9	2.6	2.6	0.6	11.6	12.7	4.6
D3-100-R1	2/11/2014	35.7	71	71.7	53	74.7	484	490	76.1
D3-100-R2	2/11/2014	26.9	62	63.9	39	62.9	449	436	73.9
D3-100-R3	2/11/2014	29.1	69	72.6	47	68.1	477	481	71.8
Mean		30.6	67	69.4	46	68.6	470	469	73.9
StDev		4.6	4.7	4.8	7.0	5.9	18.5	28.9	2.2
%CV		15.0	7.0	6.9	15.2	8.6	3.9	6.2	3.0
D3-50-R1	2/11/2014	27.7	33	70.2	22	66.7	508	388	63.0
D3-50-R2	2/11/2014	21.0	32	71.1	18	56.3	518	499	57.6
D3-50-R3	2/11/2014	28.4	36	72.0	19	52.8	335	365	42.9
Mean		25.7	34	71.1	20	58.6	454	417	54.5
StDev		4.1	2.1	0.9	2.1	7.2	102.9	71.7	10.4
%CV		16.0	6.2	1.3	10.5	12.3	22.7	17.2	19.1

Appendix L: Table 1, Linearity – Healthy Donors 1-3 – Continued

Table 1 Continued	Acquisition Date	%tSTAT3 unstim [CD3+/CD4+ Lymphs]	MESF tSTAT3 unstim [CD3+ Lymphs]	MESF tSTAT3 unstim [CD3+/CD4+ Lymphs]	%pSTAT3 unstim [CD3+ Lymphs]	%pSTAT3 unstim [CD3+/CD4+ Lymphs]	MESF pSTAT3 unstim [CD3+ Lymphs]	MESF pSTAT3 unstim [CD3+/CD4+ Lymphs]	MESF FMO-PE STIM [CD3+ Lymphs]	MESF FMO-PE STIM [CD3+/CD4+ Lymphs]
D3-2000-R1	2/11/2014	93.6	2601	2660	48.8	65.8	1630	2002	385	391
D3-2000-R2	2/11/2014	93.0	2508	2568	51.6	66.8	1720	2082	410	430
D3-2000-R3	2/11/2014	92.5	2503	2521	50.1	65.4	1659	2023	383	374
Mean		93.0	2537	2583	50.2	66.0	1670	2036	393	398
StDev		0.6	55.2	70.7	1.4	0.7	45.9	41.5	15.0	28.7
%CV		0.6	2.2	2.7	2.8	1.1	2.7	2.0	3.8	7.2
D3-1000-R1	2/11/2014	91.5	2561	2576	46.7	62.0	1533	1827	385	388
D3-1000-R2	2/11/2014	90.5	2447	2470	49.3	62.9	1615	1904	420	411
D3-1000-R3	2/11/2014	89.5	2384	2400	49.9	64.6	1658	2016	454	453
Mean		90.5	2464	2482	48.6	63.2	1602	1916	420	417
StDev		1.0	89.7	88.6	1.7	1.3	63.5	95.0	34.5	33.0
%CV		1.1	3.6	3.6	3.5	2.1	4.0	5.0	8.2	7.9
D3-500-R1	2/11/2014	89.8	2347	2351	46.1	63.5	1555	1937	429	426
D3-500-R2	2/11/2014	85.9	2397	2385	50.6	64.5	1666	2010	473	457
D3-500-R3	2/11/2014	84.6	2255	2255	46.7	59.4	1557	1887	419	409
Mean		86.8	2333	2330	47.8	62.5	1593	1945	440	431
StDev		2.7	72.0	67.4	2.4	2.7	63.5	61.9	28.7	24.3
%CV		3.1	3.1	2.9	5.0	4.3	4.0	3.2	6.5	5.6
D3-300-R1	2/11/2014	84.2	2230	2144	43.0	55.7	1437	1656	430	430
D3-300-R2	2/11/2014	79.4	2197	2229	49.5	64.5	1656	1991	419	402
D3-300-R3	2/11/2014	81.0	2177	2197	41.7	56.7	1452	1646	488	518
Mean		81.5	2201	2190	44.7	59.0	1515	1764	446	450
StDev		2.4	26.8	42.9	4.2	4.8	122.3	196.4	37.1	60.5
%CV		2.9	1.2	2.0	9.4	8.1	8.1	11.1	8.3	13.4
D3-100-R1	2/11/2014	77.6	2111	2118	53.1	66.7	1861	1878	433	433
D3-100-R2	2/11/2014	76.1	2047	2053	57.1	66.0	1755	1827	362	395
D3-100-R3	2/11/2014	83.0	2031	2049	44.6	56.5	1464	1707	349	347
Mean		78.9	2063	2073	51.6	63.1	1693	1804	381	392
StDev		3.6	42.3	38.7	6.4	5.7	205.6	87.8	45.2	43.1
%CV		4.6	2.1	1.9	12.4	9.0	12.1	4.9	11.9	11.0
D3-50-R1	2/11/2014	68.4	1844	1850	41.9	65.0	1217	1907	376	376
D3-50-R2	2/11/2014	66.7	1739	1821	43.3	73.7	1420	2066	388	452
D3-50-R3	2/11/2014	81.8	1635	1844	44.8	61.9	1576	1697	510	589
Mean		72.3	1739	1838	43.3	66.9	1404	1890	425	472
StDev		8.3	104.5	15.3	1.5	6.1	180.0	185.1	74.1	107.9
%CV		11.5	6.0	0.8	3.5	9.1	12.8	9.8	17.4	22.9

Appendix M: Table 1, Linearity – Healthy Donors 1-3 – Continued

Table 1 Continued	Acquisition Date	%tSTAT3 STIM [CD3+ Lymphs]	%tSTAT3 STIM [CD3+/CD4+ Lymphs]	MESF tSTAT3 STIM [CD3+ Lymphs]	MESF tSTAT3 STIM [CD3+/CD4+ Lymphs]	%pSTAT3 STIM [CD3+ Lymphs]	%pSTAT3 STIM [CD3+/CD4+ Lymphs]	MESF pSTAT3 STIM [CD3+ Lymphs]	MESF pSTAT3 STIM [CD3+/CD4+ Lymphs]
D3-2000-R1	2/11/2014	88.5	92.2	2591	2604	86.6	98.5	13232	15041
D3-2000-R2	2/11/2014	88.0	91.5	2612	2606	88.9	98.6	13759	15182
D3-2000-R3	2/11/2014	89.3	92.3	2522	2549	88.9	98.4	13719	14997
Mean		88.6	92.0	2575	2586	88.1	98.5	13570	15073
StDev		0.7	0.4	47.1	32.3	1.3	0.1	293.4	96.6
%CV		0.8	0.4	1.8	1.2	1.5	0.1	2.2	0.6
D3-1000-R1	2/11/2014	89.1	91.3	2527	2540	89.2	98.8	13894	15253
D3-1000-R2	2/11/2014	87.9	92.9	2431	2432	88.2	98.7	12866	14655
D3-1000-R3	2/11/2014	85.8	90.0	2368	2361	87.7	99.3	13371	14937
Mean		87.6	91.4	2442	2444	88.4	98.9	13377	14948
StDev		1.7	1.5	80.1	90.1	0.8	0.3	514.0	299.2
%CV		1.9	1.6	3.3	3.7	0.9	0.3	3.8	2.0
D3-500-R1	2/11/2014	83.0	86.2	2448	2462	87.7	97.8	12512	14052
D3-500-R2	2/11/2014	82.4	88.4	2334	2415	90.8	98.2	12682	13322
D3-500-R3	2/11/2014	80.4	86.8	2240	2273	86.5	98.2	12939	14476
Mean		81.9	87.1	2341	2383	88.3	98.1	12711	13950
StDev		1.4	1.1	104.2	98.4	2.2	0.2	215.0	583.7
%CV		1.7	1.3	4.5	4.1	2.5	0.2	1.7	4.2
D3-300-R1	2/11/2014	81.8	86.9	2252	2269	89.8	98.6	13095	14974
D3-300-R2	2/11/2014	81.5	87.1	2204	2308	85.3	98.6	11780	12722
D3-300-R3	2/11/2014	70.6	80.3	2167	2197	88.7	99.3	11889	12636
Mean		78.0	84.8	2208	2258	87.9	98.8	12255	13444
StDev		6.4	3.9	42.6	56.3	2.3	0.4	729.8	1325.7
%CV		8.2	4.6	1.9	2.5	2.6	0.4	6.0	9.9
D3-100-R1	2/11/2014	75.0	87.0	2053	1979	90.1	100.0	12148	13038
D3-100-R2	2/11/2014	63.1	70.7	1991	2066	91.9	97.4	13322	13738
D3-100-R3	2/11/2014	71.9	77.5	2047	2053	88.4	97.9	12261	13119
Mean		70.0	78.4	2030	2033	90.1	98.4	12577	13298
StDev		6.2	8.2	34.2	46.9	1.8	1.4	647.7	382.9
%CV		8.9	10.5	1.7	2.3	2.0	1.4	5.1	2.9
D3-50-R1	2/11/2014	66.7	79.2	1750	1931	84.9	95.5	11009	11077
D3-50-R2	2/11/2014	58.1	83.3	1805	2105	81.3	100.0	10039	12374
D3-50-R3	2/11/2014	73.3	68.4	1907	1967	83.3	100.0	6527	9886
Mean		66.0	77.0	1821	2001	83.2	98.5	9192	11112
StDev		7.6	7.7	79.7	91.8	1.8	2.6	2358.1	1244.4
%CV		11.5	10.0	4.4	4.6	2.2	2.6	25.7	11.2

Appendix N: Table 1, Linearity – Healthy Donors 1-3 – **Continued**

Table 1 Continued	Acquisition Date	MFI FMO-PE unstim [CD3+ Lymphs]	MFI FMO-PE unstim [CD3+/CD4+ Lymphs]	MFI tSTAT3 unstim [CD3+ Lymphs]	MFI tSTAT3 unstim [CD3+/CD4+ Lymphs]	MFI pSTAT3 unstim [CD3+ Lymphs]	MFI pSTAT3 unstim [CD3+/CD4+ Lymphs]
D3-2000-R1	2/11/2014	150	147	967	989	609	746
D3-2000-R2	2/11/2014	157	156	933	955	642	776
D3-2000-R3	2/11/2014	158	157	931	938	620	754
Mean		155	153	944	961	624	759
StDev		4.4	5.5	20.2	26.0	16.8	15.5
%CV		2.8	3.6	2.1	2.7	2.7	2.0
D3-1000-R1	2/11/2014	173	166	953	958	573	682
D3-1000-R2	2/11/2014	162	163	911	919	604	710
D3-1000-R3	2/11/2014	165	158	887	893	619	752
Mean		167	162	917	923	599	715
StDev		5.7	4.0	33.4	32.7	23.5	35.2
%CV		3.4	2.5	3.6	3.5	3.9	4.9
D3-500-R1	2/11/2014	164	152	874	875	581	722
D3-500-R2	2/11/2014	162	165	892	888	622	749
D3-500-R3	2/11/2014	171	177	840	840	582	704
Mean		166	165	869	868	595	725
StDev		4.7	12.5	26.4	24.8	23.4	22.6
%CV		2.8	7.6	3.0	2.9	3.9	3.1
D3-300-R1	2/11/2014	150	143	831	799	538	618
D3-300-R2	2/11/2014	189	184	818	830	618	742
D3-300-R3	2/11/2014	170	171	811	818	543	615
Mean		170	166	820	816	566	658
StDev		19.5	21.0	10.1	15.6	44.8	72.5
%CV		11.5	12.7	1.2	1.9	7.9	11.0
D3-100-R1	2/11/2014	183	185	787	789	694	701
D3-100-R2	2/11/2014	170	165	763	765	655	682
D3-100-R3	2/11/2014	180	182	757	764	547	638
Mean		178	177	769	773	632	674
StDev		6.8	10.8	15.9	14.2	76.2	32.3
%CV		3.8	6.1	2.1	1.8	12.1	4.8
D3-50-R1	2/11/2014	192	147	688	690	456	711
D3-50-R2	2/11/2014	196	189	649	680	531	770
D3-50-R3	2/11/2014	127	138	611	688	589	634
Mean		172	158	649	686	525	705
StDev		38.7	27.2	38.5	5.3	66.7	68.2
%CV		22.5	17.2	5.9	0.8	12.7	9.7

Appendix O: Table 1, Linearity – Healthy Donors 1-3 – Continued

Table 1 Continued	Acquisition Date	MFI FMO-PE STIM [CD3+ Lymphs]	MFI FMO-PE STIM [CD3+/CD4+ Lymphs]	MFI tSTAT3 STIM [CD3+ Lymphs]	MFI tSTAT3 STIM [CD3+/CD4+ Lymphs]	MFI pSTAT3 STIM [CD3+ Lymphs]	MFI pSTAT3 STIM [CD3+/CD4+ Lymphs]
D3-2000-R1	2/11/2014	146	148	964	968	4845	5500
D3-2000-R2	2/11/2014	155	163	971	969	5036	5552
D3-2000-R3	2/11/2014	145	142	938	948	5021	5484
Mean		149	151	958	962	4967	5512
StDev		5.5	10.8	17.4	11.8	106.2	35.6
%CV		3.7	7.2	1.8	1.2	2.1	0.6
D3-1000-R1	2/11/2014	146	147	940	945	5085	5577
D3-1000-R2	2/11/2014	159	156	905	905	4712	5361
D3-1000-R3	2/11/2014	172	171	881	879	4895	5463
Mean		159	158	909	910	4897	5467
StDev		13.0	12.1	29.7	33.2	186.5	108.1
%CV		8.2	7.7	3.3	3.6	3.8	2.0
D3-500-R1	2/11/2014	162	161	911	916	4584	5142
D3-500-R2	2/11/2014	179	173	869	899	4646	4878
D3-500-R3	2/11/2014	159	155	834	846	4738	5296
Mean		167	163	871	887	4656	5105
StDev		10.8	9.2	38.6	36.5	77.5	211.4
%CV		6.5	5.6	4.4	4.1	1.7	4.1
D3-300-R1	2/11/2014	163	163	839	845	4795	5476
D3-300-R2	2/11/2014	158	152	821	859	4318	4660
D3-300-R3	2/11/2014	184	196	807	818	4358	4629
Mean		168	170	822	841	4490	4922
StDev		13.8	22.9	16.0	20.8	264.6	480.3
%CV		8.2	13.5	1.9	2.5	5.9	9.8
D3-100-R1	2/11/2014	164	164	765	738	4452	4775
D3-100-R2	2/11/2014	137	150	742	770	4878	5028
D3-100-R3	2/11/2014	132	132	763	765	4492	4804
Mean		144	149	757	758	4607	4869
StDev		17.2	16.0	12.7	17.2	235.3	138.5
%CV		11.9	10.7	1.7	2.3	5.1	2.8
D3-50-R1	2/11/2014	142	142	653	720	4038	4063
D3-50-R2	2/11/2014	147	171	673	784	3686	4534
D3-50-R3	2/11/2014	193	222	711	733	2406	3630
Mean		161	178	679	746	3377	4076
StDev		28.1	40.5	29.5	33.8	858.8	452.1
%CV		17.5	22.8	4.3	4.5	25.4	11.1

Appendix P: Table 2, Linearity – Relative Error Calculations – Donor 1

Table 2: Donor 1 Linearity (%RE cutoff is ≥25%)	%tSTAT3 unstim [CD3+ Lymphs]	%tSTAT3 unstim [CD3+/CD4+ Lymphs]	MESF tSTAT3 unstim [CD3+ Lymphs]	MESF tSTAT3 unstim [CD3+/CD4+ Lymphs]	%pSTAT3 unstim [CD3+ Lymphs]	%pSTAT3 unstim [CD3+/CD4+ Lymphs]	MESF pSTAT3 unstim [CD3+ Lymphs]	MESF pSTAT3 unstim [CD3+/CD4+ Lymphs]
Accepted Value (AVG @2000 spike)	38.7	50.5	1633	1634	2.4	5.9	735	794
%Relative Error- 1000 spike	8.5	12.9	3.4	4.5	12.5	11.9	2.2	3.0
%Relative Error- 500 spike	23.0	22.2	8.0	9.8	4.2	6.8	0.3	2.4
%Relative Error- 300 spike	35.1	24.6	10.6	10.2	33.3	15.3	2.2	0.4
%Relative Error- 100 spike	51.7	42.4	15.0	14.9	37.5	16.9	3.5	0.4
%Relative Error- 50 spike	51.9	27.7	17.5	10.5	16.7	8.5	3.8	3.4

Table 2: Donor 1 Linearity (%RE cutoff is ≥25%) - Continued	%tSTAT3 STIM [CD3+ Lymphs]	%tSTAT3 STIM [CD3+/CD4+ Lymphs]	MESF tSTAT3 STIM [CD3+ Lymphs]	MESF tSTAT3 STIM [CD3+/CD4+ Lymphs]	%pSTAT3 STIM [CD3+ Lymphs]	%pSTAT3 STIM [CD3+/CD4+ Lymphs]	MESF pSTAT3 STIM [CD3+ Lymphs]	MESF pSTAT3 STIM [CD3+/CD4+ Lymphs]	MFI FMO-PE unstim [CD3+ Lymphs]	MFI FMO-PE unstim [CD3+/CD4+ Lymphs]
Accepted Value (AVG @2000 spike)	36.6	46.6	1606	1583	54.2	98.4	2822	10649	217	209
%Relative Error- 1000 spike	12.6	11.6	4.2	3.8	0.7	1.5	1.2	2.7	1.4	3.3
%Relative Error- 500 spike	26.0	30.7	7.5	10.7	2.0	1.1	5.2	6.1	5.5	8.1
%Relative Error- 300 spike	29.8	29.8	9.7	9.5	0.2	0.4	0.2	7.4	3.2	14.8
%Relative Error- 100 spike	32.8	33.5	13.8	11.7	11.1	1.4	4.7	17.2	6.9	10.0
%Relative Error- 50 spike	59.6	33.7	19.6	17.4	10.3	2.2	54.8	13.1	4.1	19.6

Table 2: Donor 1 Linearity (%RE cutoff is ≥25%) - Continued	MFI tSTAT3 unstim [CD3+ Lymphs]	MFI tSTAT3 unstim [CD3+/CD4+ Lymphs]	MFI pSTAT3 unstim [CD3+ Lymphs]	MFI pSTAT3 unstim [CD3+/CD4+ Lymphs]	MFI FMO-PE STIM [CD3+ Lymphs]	MFI FMO-PE STIM [CD3+/CD4+ Lymphs]	MFI tSTAT3 STIM [CD3+ Lymphs]	MFI tSTAT3 STIM [CD3+/CD4+ Lymphs]	MFI pSTAT3 STIM [CD3+ Lymphs]	MFI pSTAT3 STIM [CD3+/CD4+ Lymphs]
Accepted Value (AVG @2000 spike)	651	651	299	322	215	214	641	631	1111	4064
%Relative Error- 1000 spike	3.4	4.3	2.0	2.8	3.7	1.4	4.2	3.6	1.2	2.6
%Relative Error- 500 spike	7.8	9.5	0.3	2.2	7.4	8.4	7.3	10.3	4.8	6.0
%Relative Error- 300 spike	10.4	10.0	2.0	0.3	8.8	6.5	9.5	9.2	0.3	7.3
%Relative Error- 100 spike	14.7	14.4	3.7	0.3	0.5	14.0	13.6	11.4	5.0	16.8
%Relative Error- 50 spike	17.1	10.3	3.3	3.4	23.3	4.7	19.2	17.1	51.9	32.1

Appendix Q: Table 3, Linearity – Relative Error Calculations – Donor 2

Table 3: Donor 2 Linearity (%RE cutoff is ≥25%)	%tSTAT3 unstim [CD3+ Lymphs]	%tSTAT3 unstim [CD3+/CD4+ Lymphs]	MESF tSTAT3 unstim [CD3+ Lymphs]	MESF tSTAT3 unstim [CD3+/CD4+ Lymphs]	%pSTAT3 unstim [CD3+ Lymphs]	%pSTAT3 unstim [CD3+/CD4+ Lymphs]	MESF pSTAT3 unstim [CD3+ Lymphs]	MESF pSTAT3 unstim [CD3+/CD4+ Lymphs]
Accepted Value (AVG @2000 spike)	86.2	80.6	1797	1790	49.8	51.7	1228	1399
%Relative Error- 1000 spike	3.6	4.2	6.5	5.9	4.6	3.3	3.2	3.3
%Relative Error- 500 spike	19.8	27.0	18.4	17.6	1.4	2.5	0.4	1.9
%Relative Error- 300 spike	29.8	37.7	24.4	23.3	0.8	1.9	0.6	2.3
%Relative Error- 100 spike	39.1	44.0	28.9	26.1	9.0	11.8	6.8	12.6
%Relative Error- 50 spike	42.2	49.1	31.1	29.1	21.3	16.1	9.5	16.0

Table 3: Donor 2 Linearity (%RE cutoff is ≥25%) - Continued	%tSTAT3 STIM [CD3+ Lymphs]	%tSTAT3 STIM [CD3+/CD4+ Lymphs]	MESF tSTAT3 STIM [CD3+ Lymphs]	MESF tSTAT3 STIM [CD3+/CD4+ Lymphs]	%pSTAT3 STIM [CD3+ Lymphs]	%pSTAT3 STIM [CD3+/CD4+ Lymphs]	MESF pSTAT3 STIM [CD3+ Lymphs]	MESF pSTAT3 STIM [CD3+/CD4+ Lymphs]	MFI FMO-PE unstim [CD3+ Lymphs]	MFI FMO-PE unstim [CD3+/CD4+ Lymphs]
Accepted Value (AVG @2000 spike)	81.3	72.7	1682	1674	90.1	99.2	13046	13830	173	171
%Relative Error- 1000 spike	5.8	8.9	6.0	6.7	0.7	0.6	3.4	3.1	1.2	1.8
%Relative Error- 500 spike	13.0	15.8	11.2	10.5	0.1	0.0	8.9	7.8	1.2	1.2
%Relative Error- 300 spike	20.4	24.3	16.4	15.8	0.7	1.7	13.7	13.4	11.6	17.5
%Relative Error- 100 spike	22.3	25.9	19.3	18.3	0.3	0.2	14.3	14.2	16.2	22.8
%Relative Error- 50 spike	37.4	50.1	25.6	30.4	0.2	0.5	12.1	15.5	25.4	17.0

Table 3: Donor 2 Linearity (%RE cutoff is ≥25%) - Continued	MFI tSTAT3 unstim [CD3+ Lymphs]	MFI tSTAT3 unstim [CD3+/CD4+ Lymphs]	MFI pSTAT3 unstim [CD3+ Lymphs]	MFI pSTAT3 unstim [CD3+/CD4+ Lymphs]	MFI FMO-PE STIM [CD3+ Lymphs]	MFI FMO-PE STIM [CD3+/CD4+ Lymphs]	MFI tSTAT3 STIM [CD3+ Lymphs]	MFI tSTAT3 STIM [CD3+/CD4+ Lymphs]	MFI pSTAT3 STIM [CD3+ Lymphs]	MFI pSTAT3 STIM [CD3+/CD4+ Lymphs]
Accepted Value (AVG @2000 spike)	715	712	493	560	183	179	670	667	4956	5246
%Relative Error- 1000 spike	6.4	5.8	3.2	3.4	0.5	1.1	5.8	6.6	3.3	3.0
%Relative Error- 500 spike	18.0	17.3	0.4	2.0	1.1	0.6	10.9	10.3	8.7	7.6
%Relative Error- 300 spike	23.9	22.8	0.6	2.1	8.7	8.9	16.1	15.6	13.4	13.1
%Relative Error- 100 spike	28.4	25.6	6.7	12.3	4.9	8.9	18.8	18.0	14.1	13.9
%Relative Error- 50 spike	30.5	28.5	9.3	15.7	22.4	11.7	25.1	29.8	11.8	15.1

Appendix R: Table 4, Linearity – Relative Error Calculations – Donor 3

Table 4: Donor 3 Linearity (%RE cutoff is ≥25%)	%tSTAT3 unstim [CD3+ Lymphs]	%tSTAT3 unstim [CD3+/CD4+ Lymphs]	MESF tSTAT3 unstim [CD3+ Lymphs]	MESF tSTAT3 unstim [CD3+/CD4+ Lymphs]	%pSTAT3 unstim [CD3+ Lymphs]	%pSTAT3 unstim [CD3+/CD4+ Lymphs]	MESF pSTAT3 unstim [CD3+ Lymphs]	MESF pSTAT3 unstim [CD3+/CD4+ Lymphs]
Accepted Value (AVG @2000 spike)	88.5	93.0	2537	2583	50.2	66.9	1670	2036
%Relative Error- 1000 spike	3.3	2.7	2.9	3.9	3.2	4.2	4.1	5.9
%Relative Error- 500 spike	6.3	6.7	8.0	9.8	4.8	5.3	4.6	4.5
%Relative Error- 300 spike	14.6	12.4	13.2	15.2	11.0	10.6	9.3	13.4
%Relative Error- 100 spike	16.5	15.2	18.7	19.7	2.8	4.4	1.4	11.4
%Relative Error- 50 spike	38.4	22.3	31.5	28.8	13.7	1.4	15.9	7.2

Table 4: Donor 3 Linearity (%RE cutoff is ≥25%) - Continued	%tSTAT3 STIM [CD3+ Lymphs]	%tSTAT3 STIM [CD3+/CD4+ Lymphs]	MESF tSTAT3 STIM [CD3+ Lymphs]	MESF tSTAT3 STIM [CD3+/CD4+ Lymphs]	%pSTAT3 STIM [CD3+ Lymphs]	%pSTAT3 STIM [CD3+/CD4+ Lymphs]	MESF pSTAT3 STIM [CD3+ Lymphs]	MESF pSTAT3 STIM [CD3+/CD4+ Lymphs]	MFI FMO-PE unstim [CD3+ Lymphs]	MFI FMO-PE unstim [CD3+/CD4+ Lymphs]
Accepted Value (AVG @2000 spike)	88.6	92.0	2575	2586	88.1	98.5	13570	15073	155	153
%Relative Error- 1000 spike	1.1	0.7	5.2	5.5	0.3	0.4	1.4	0.8	7.7	5.9
%Relative Error- 500 spike	7.6	5.3	9.1	7.8	0.2	0.4	6.3	7.5	7.1	7.8
%Relative Error- 300 spike	12.0	7.8	14.3	12.7	0.2	0.3	9.7	10.8	9.7	8.5
%Relative Error- 100 spike	21.0	14.8	21.2	21.4	2.3	0.1	7.3	11.8	14.8	15.7
%Relative Error- 50 spike	25.5	16.3	29.3	22.6	5.6	0.0	32.3	26.3	11.0	3.3

Table 4: Donor 3 Linearity (%RE cutoff is ≥25%) - Continued	MFI tSTAT3 unstim [CD3+ Lymphs]	MFI tSTAT3 unstim [CD3+/CD4+ Lymphs]	MFI pSTAT3 unstim [CD3+ Lymphs]	MFI pSTAT3 unstim [CD3+/CD4+ Lymphs]	MFI FMO-PE STIM [CD3+ Lymphs]	MFI FMO-PE STIM [CD3+/CD4+ Lymphs]	MFI tSTAT3 STIM [CD3+ Lymphs]	MFI tSTAT3 STIM [CD3+/CD4+ Lymphs]	MFI pSTAT3 STIM [CD3+ Lymphs]	MFI pSTAT3 STIM [CD3+/CD4+ Lymphs]
Accepted Value (AVG @2000 spike)	944	961	624	759	149	151	958	962	4967	5512
%Relative Error- 1000 spike	2.9	4.0	4.0	5.8	6.7	4.6	5.1	5.4	1.4	0.8
%Relative Error- 500 spike	7.9	9.7	4.6	4.5	12.1	7.9	9.1	7.8	6.3	7.4
%Relative Error- 300 spike	13.1	15.1	9.3	13.3	12.8	12.6	14.2	12.6	9.6	10.7
%Relative Error- 100 spike	18.5	19.6	1.3	11.2	3.4	1.3	21.0	21.2	7.2	11.7
%Relative Error- 50 spike	31.3	28.6	15.9	7.1	8.1	17.9	29.1	22.5	32.0	26.1

Appendix S: Table 5, Inter/Intra-Assay Precision

Table 5 - inter/Intra-Assay	Acquisition Date	%Lymphs	Region Events CD3+ Lymphs	%CD3+ [Lymphs]	Region Events CD3+/CD4+ Lymphs	%CD3+/CD4 [Lymphs]	MESF FMO-PE unstim [CD3+ Lymphs]	MESF FMO-PE unstim [CD3+/CD4+ Lymphs]	%tSTAT3 unstim [CD3+ Lymphs]	%tSTAT3 unstim [CD3+/CD4 + Lymphs]	MESF tSTAT3 unstim [CD3+ Lymphs]	MESF tSTAT3 unstim [CD3+/CD4 + Lymphs]
D1-O1-R1	2/18/2014	21.1	1342	68.9	1002	74.7	629	643	73.7	78.7	3849	3870
D1-O1-R2	2/18/2014	20.9	1296	66.6	903	69.7	698	695	70.6	77.9	3786	3785
D1-O1-R3	2/18/2014	21.0	1305	67.8	926	71.0	700	708	69.2	78.1	3711	3763
Mean-D1-Op1		21.0	1314	67.8	944	71.8	676	682	71.2	78.2	3782	3806
StDev-D1-Op1		0.1	24.4	1.2	51.8	2.6	40.4	34.4	2.3	0.4	69.1	56.5
%CV-D1-Op1		0.5	1.9	1.8	5.5	3.6	6.0	5.0	3.2	0.5	1.8	1.5
D1-O2-R1	2/18/2014	17.7	1384	71.1	1011	73.1	831	820	64.7	74.4	3513	3567
D1-O2-R2	2/18/2014	17.7	1410	71.6	1003	71.1	850	861	64.7	74.0	3561	3585
D1-O2-R3	2/18/2014	18.6	1367	70.2	960	70.2	858	868	59.6	69.4	3410	3444
Mean-D1-Op2		18.0	1387	71.0	991	71.5	846	850	63.0	72.6	3495	3532
StDev-D1-Op2		0.5	21.7	0.7	27.4	1.5	13.9	25.9	2.9	2.8	77.2	76.7
%CV-D1-Op2		2.8	1.6	1.0	2.8	2.1	1.6	3.0	4.6	3.9	2.2	2.2
Mean-Inter-Op-Donor1		19.5	1351	69.4	968	71.7	761	766	67.1	75.4	3639	3669
StDev-Inter-Op-Donor1		2.1	51.6	2.3	33.2	0.2	120.2	118.8	5.8	4.0	202.9	193.7
%CV-Inter-Op-Donor1		10.8	3.8	3.3	3.4	0.3	15.8	15.5	8.6	5.3	5.6	5.3
D2-O1-R1	2/18/2014	32.6	1494	71.9	804	53.8	765	714	54.9	66.2	3221	3248
D2-O1-R2	2/18/2014	34.0	1510	72.7	785	52.0	760	736	55.9	66.2	3228	3218
D2-O1-R3	2/18/2014	32.7	1495	72.6	764	51.1	738	741	51.4	61.6	3142	3187
Mean-D2-Op1		33.1	1500	72.4	784	52.3	754	730	54.1	64.7	3197	3218
StDev-D2-Op1		0.8	9.0	0.4	20.0	1.4	14.4	14.4	2.4	2.7	47.8	30.5
%CV-D2-Op1		2.4	0.6	0.6	2.6	2.7	1.9	2.0	4.4	4.2	1.5	0.9
D2-O2-R1	2/18/2014	31.9	1449	70.3	767	52.9	758	723	45.1	53.1	2914	2889
D2-O2-R2	2/18/2014	33.6	1475	72.1	788	53.4	781	751	45.9	59.5	2990	3023
D2-O2-R3	2/18/2014	32.8	1482	72.1	821	55.4	787	767	48.5	60.2	3041	3032
Mean-D2-Op2		32.8	1469	71.5	792	53.9	775	747	46.5	57.6	2982	2981
StDev-D2-Op2		0.9	17.4	1.0	27.2	1.3	15.3	22.3	1.8	3.9	63.9	80.1
%CV-D2-Op2		2.7	1.2	1.4	3.4	2.4	2.0	3.0	3.9	6.8	2.1	2.7
Mean-Inter-Op-Donor2		33.0	1485	72.0	788	53.1	765	739	50.3	61.2	3090	3100
StDev-Inter-Op-Donor2		0.2	21.9	0.6	5.7	1.1	14.8	12.0	5.4	5.0	152.0	167.6
%CV-Inter-Op-Donor2		0.6	1.5	0.8	0.7	2.1	1.9	1.6	10.7	8.2	4.9	5.4

Appendix T: Table 5, Inter/Intra-Assay Precision – Continued

Table 5 - inter/Intra-Assay	Acquisition Date	%pSTAT3 unstim [CD3+ Lymphs]	%pSTAT3 unstim [CD3+/CD4+ Lymphs]	MESF pSTAT3 unstim [CD3+ Lymphs]	MESF pSTAT3 unstim [CD3+/CD4+ Lymphs]	MESF FMO-PE STIM [CD3+ Lymphs]	MESF FMO-PE STIM [CD3+/CD4+ Lymphs]
D1-O1-R1	2/18/2014	35.2	47.9	2253	2706	687	677
D1-O1-R2	2/18/2014	37.1	50.3	2361	2843	711	696
D1-O1-R3	2/18/2014	37.0	49.7	2431	2796	743	701
Mean-D1-Op1		36.4	49.3	2348	2782	714	691
StDev-D1-Op1		1.1	1.2	89.7	69.6	28.1	12.7
%CV-D1-Op1		3.0	2.4	3.8	2.5	3.9	1.8
D1-O2-R1	2/18/2014	40.9	53.4	2594	3029	638	642
D1-O2-R2	2/18/2014	39.3	52.0	2485	2938	679	683
D1-O2-R3	2/18/2014	40.3	52.8	2530	2975	658	639
Mean-D1-Op2		40.2	52.7	2536	2981	658	655
StDev-D1-Op2		0.8	0.7	54.8	45.8	20.5	24.6
%CV-D1-Op2		2.0	1.3	2.2	1.5	3.1	3.8
Mean-Inter-Op-Donor1		38.3	51.0	2442	2882	686	673
StDev-Inter-Op-Donor1		2.7	2.4	132.9	140.7	39.6	25.5
%CV-Inter-Op-Donor1		7.0	4.7	5.4	4.9	5.8	3.8
D2-O1-R1	2/18/2014	3.2	8.0	1176	1244	790	740
D2-O1-R2	2/18/2014	3.0	8.1	1217	1318	780	775
D2-O1-R3	2/18/2014	2.8	6.2	1193	1280	813	825
Mean-D2-Op1		3.0	7.4	1195	1281	794	780
StDev-D2-Op1		0.2	1.1	20.6	37.0	16.9	42.7
%CV-D2-Op1		6.7	14.9	1.7	2.9	2.1	5.5
D2-O2-R1	2/18/2014	6.3	14.5	1279	1405	762	741
D2-O2-R2	2/18/2014	6.3	12.9	1242	1411	746	723
D2-O2-R3	2/18/2014	6.4	15.1	1308	1508	779	765
Mean-D2-Op2		6.3	14.2	1276	1441	762	743
StDev-D2-Op2		0.1	1.1	33.1	57.8	16.5	21.1
%CV-D2-Op2		1.6	7.7	2.6	4.0	2.2	2.8
Mean-Inter-Op-Donor2		4.7	10.8	1236	1361	778	762
StDev-Inter-Op-Donor2		2.3	4.8	57.3	113.1	22.6	26.2
%CV-Inter-Op-Donor2		48.9	44.4	4.6	8.3	2.9	3.4

Appendix U: Table 5, Inter/Intra-Assay Precision – Continued

Table 5 - inter/Intra-Assay	Acquisition Date	%tSTAT3 STIM [CD3+ Lymphs]	%tSTAT3 STIM [CD3+/CD4+ Lymphs]	MESF tSTAT3 STIM [CD3+ Lymphs]	MESF tSTAT3 STIM [CD3+/CD4+ Lymphs]	%pSTAT3 STIM [CD3+ Lymphs]	%pSTAT3 STIM [CD3+/CD4+ Lymphs]
D1-O1-R1	2/18/2014	63.3	74.8	3528	3588	88.6	97.4
D1-O1-R2	2/18/2014	63.6	71.6	3538	3551	86.8	96.8
D1-O1-R3	2/18/2014	57.0	65.8	3340	3343	87.4	96.9
Mean-D1-Op1		61.3	70.7	3469	3494	87.6	97.0
StDev-D1-Op1		3.7	4.6	111.5	132.1	0.9	0.3
%CV-D1-Op1		6.0	6.5	3.2	3.8	1.0	0.3
D1-O2-R1	2/18/2014	46.7	58.1	3003	3003	89.5	98.1
D1-O2-R2	2/18/2014	46.4	57.7	3001	3005	89.0	98.0
D1-O2-R3	2/18/2014	46.7	57.8	2976	3073	88.8	98.1
Mean-D1-Op2		46.6	57.9	2993	3027	89.1	98.1
StDev-D1-Op2		0.2	0.2	15.0	39.8	0.4	0.1
%CV-D1-Op2		0.4	0.3	0.5	1.3	0.4	0.1
Mean-Inter-Op-Donor1		54.0	64.3	3231	3261	88.4	97.6
StDev-Inter-Op-Donor1		10.4	9.1	336.6	330.2	1.1	0.8
%CV-Inter-Op-Donor1		19.3	14.2	10.4	10.1	1.2	0.8
D2-O1-R1	2/18/2014	39.6	49.6	2832	2794	46.9	71.1
D2-O1-R2	2/18/2014	37.0	47.9	2769	2770	44.8	68.0
D2-O1-R3	2/18/2014	41.2	51.4	2856	2842	46.0	70.0
Mean-D2-Op1		39.3	49.6	2819	2802	45.9	69.7
StDev-D2-Op1		2.1	1.8	44.9	36.7	1.1	1.6
%CV-D2-Op1		5.3	3.6	1.6	1.3	2.4	2.3
D2-O2-R1	2/18/2014	35.1	43.8	2678	2654	47.3	72.1
D2-O2-R2	2/18/2014	37.4	45.6	2713	2717	48.1	68.8
D2-O2-R3	2/18/2014	33.8	44.3	2666	2667	47.3	69.1
Mean-D2-Op2		35.4	44.6	2686	2679	47.6	70.0
StDev-D2-Op2		1.8	0.9	24.4	33.3	0.5	1.8
%CV-D2-Op2		5.1	2.0	0.9	1.2	1.1	2.6
Mean-Inter-Op-Donor2		37.4	47.1	2753	2741	46.8	69.9
StDev-Inter-Op-Donor2		2.8	3.5	94.0	87.0	1.2	0.2
%CV-Inter-Op-Donor2		7.5	7.4	3.4	3.2	2.6	0.3

Appendix V: Table 5, Inter/Intra-Assay Precision – Continued

Table 5 - inter/Intra-Assay	Acquisition Date	MESF pSTAT3 STIM [CD3+ Lymphs]	MESF pSTAT3 STIM [CD3+/CD4+ Lymphs]	MFI FMO-PE unstim [CD3+ Lymphs]	MFI FMO-PE unstim [CD3+/CD4+ Lymphs]	MFI tSTAT3 unstim [CD3+ Lymphs]	MFI tSTAT3 unstim [CD3+/CD4+ Lymphs]
D1-O1-R1	2/18/2014	19904	21127	161	164	868	872
D1-O1-R2	2/18/2014	19804	21139	177	176	855	855
D1-O1-R3	2/18/2014	18073	19237	177	179	839	850
Mean-D1-Op1		19260	20501	172	173	854	859
StDev-D1-Op1		1029.5	1094.7	9.2	7.9	14.5	11.5
%CV-D1-Op1		5.3	5.3	5.3	4.6	1.7	1.3
D1-O2-R1	2/18/2014	22534	24484	208	206	797	809
D1-O2-R2	2/18/2014	22165	23891	213	215	807	812
D1-O2-R3	2/18/2014	21179	22802	214	217	775	783
Mean-D1-Op2		21959	23726	212	213	793	801
StDev-D1-Op2		700.5	853.1	3.2	5.9	16.4	15.9
%CV-D1-Op2		3.2	3.6	1.5	2.8	2.1	2.0
Mean-Inter-Op-Donor1		20610	22114	192	193	824	830
StDev-Inter-Op-Donor1		1908.5	2280.4	28.3	28.3	43.1	41.0
%CV-Inter-Op-Donor1		9.3	10.3	14.7	14.7	5.2	4.9
D2-O1-R1	2/18/2014	2511	12197	193	181	735	741
D2-O1-R2	2/18/2014	2291	12229	192	186	737	735
D2-O1-R3	2/18/2014	2344	12277	186	187	718	728
Mean-D2-Op1		2382	12234	190	185	730	735
StDev-D2-Op1		114.8	40.3	3.8	3.2	10.4	6.5
%CV-D2-Op1		4.8	0.3	2.0	1.7	1.4	0.9
D2-O2-R1	2/18/2014	2583	13085	191	183	670	664
D2-O2-R2	2/18/2014	2663	11766	196	189	686	693
D2-O2-R3	2/18/2014	2489	11976	198	193	697	695
Mean-D2-Op2		2578	12276	195	188	684	684
StDev-D2-Op2		87.1	708.7	3.6	5.0	13.6	17.3
%CV-D2-Op2		3.4	5.8	1.8	2.7	2.0	2.5
Mean-Inter-Op-Donor2		2480	12255	193	187	707	710
StDev-Inter-Op-Donor2		138.6	29.7	3.5	2.1	32.5	36.1
%CV-Inter-Op-Donor2		5.6	0.2	1.8	1.1	4.6	5.1

Appendix W: Table 5, Inter/Intra-Assay Precision – Continued

Table 5 - Inter/Intra-Assay	Acquisition Date	MFI pSTAT3 unstim [CD3+ Lymphs]	MFI pSTAT3 unstim [CD3+/CD4+ Lymphs]	MFI FMO-PE STIM [CD3+ Lymphs]	MFI FMO-PE STIM [CD3+/CD4+ Lymphs]	MFI tSTAT3 STIM [CD3+ Lymphs]	MFI tSTAT3 STIM [CD3+/CD4+ Lymphs]	MFI pSTAT3 STIM [CD3+ Lymphs]	MFI pSTAT3 STIM [CD3+/CD4+ Lymphs]
D1-O1-R1	2/18/2014	527	625	174	172	800	813	4011	4240
D1-O1-R2	2/18/2014	551	655	180	176	803	805	3992	4242
D1-O1-R3	2/18/2014	566	645	188	178	760	761	3666	3885
Mean-D1-Op1		548	642	181	175	788	793	3890	4122
StDev-D1-Op1		19.7	15.3	7.0	3.1	24.0	28.0	193.9	205.5
%CV-D1-Op1		3.6	2.4	3.9	1.8	3.0	3.5	5.0	5.0
D1-O2-R1	2/18/2014	601	694	163	164	689	689	4502	4864
D1-O2-R2	2/18/2014	577	675	173	173	688	689	4434	4754
D1-O2-R3	2/18/2014	587	683	167	163	683	704	4250	4552
Mean-D1-Op2		588	684	168	167	687	694	4395	4723
StDev-D1-Op2		12.1	9.5	5.0	5.5	3.2	8.7	130.4	158.2
%CV-D1-Op2		2.1	1.4	3.0	3.3	0.5	1.3	3.0	3.3
Mean-Inter-Op-Donor1		568	663	175	171	738	744	4143	4423
StDev-Inter-Op-Donor1		28.3	29.7	9.2	5.7	71.4	70.0	357.1	425.0
%CV-Inter-Op-Donor1		5.0	4.5	5.3	3.3	9.7	9.4	8.6	9.6
D2-O1-R1	2/18/2014	288	303	198	187	652	644	583	2542
D2-O1-R2	2/18/2014	297	320	196	195	639	639	535	2548
D2-O1-R3	2/18/2014	291	311	204	207	657	654	547	2557
Mean-D2-Op1		292	311	199	196	649	646	555	2549
StDev-D2-Op1		4.6	8.5	4.2	10.1	9.3	7.6	25.0	7.5
%CV-D2-Op1		1.6	2.7	2.1	5.2	1.4	1.2	4.5	0.3
D2-O2-R1	2/18/2014	311	339	192	187	619	614	599	2714
D2-O2-R2	2/18/2014	303	341	188	183	627	628	616	2458
D2-O2-R3	2/18/2014	318	363	196	193	617	617	578	2499
Mean-D2-Op2		311	348	192	188	621	620	598	2557
StDev-D2-Op2		7.5	13.3	4.0	5.0	5.3	7.4	19.0	137.5
%CV-D2-Op2		2.4	3.8	2.1	2.7	0.9	1.2	3.2	5.4
Mean-Inter-Op-Donor2		302	330	196	192	635	633	577	2553
StDev-Inter-Op-Donor2		13.4	26.2	4.9	5.7	19.8	18.4	30.4	5.7
%CV-Inter-Op-Donor2		4.4	7.9	2.5	3.0	3.1	2.9	5.3	0.2

Appendix X: Table 6: Assay Transfer, Flow Assay

Table 6: Assay Transfer	Acquisition Date	%Lymphs	Region Events CD3+ Lymphs	%CD3+ [Lymphs]	Region Events CD3+/CD4+ Lymphs	%CD3+/CD4 [Lymphs]	MESF FMO-PE unstim [CD3+ Lymphs]	MESF FMO-PE unstim [CD3+/CD4+ Lymphs]	%tSTAT3 unstim [CD3+ Lymphs]	%tSTAT3 unstim [CD3+/CD4+ Lymphs]
DONOR1A	4/22/2014	20.7	4214	81.1	2643	62.7	1080	1075	46.2	44.8
DONOR1B	4/22/2014	34.5	6213	79.0	4010	64.5	874	876	44.7	46.9
DONOR1C	4/22/2014	33.9	6033	79.4	3956	65.6	874	884	39.9	42.9
Mean		29.7	5487	79.8	3536	64.3	943	945	43.6	44.9
StDev		7.8	1105.8	1.1	774.1	1.5	118.9	112.7	3.3	2.0
%CV		26.3	20.2	1.4	21.9	2.3	12.6	11.9	7.6	4.5
DONOR2A	4/22/2014	27.9	4647	66.8	3447	74.2	739	743	19.0	19.0
DONOR2B	4/22/2014	27.8	4551	66.0	3369	74.0	776	768	42.7	42.2
DONOR2C	4/22/2014	26.6	5354	67.0	3967	74.1	824	811	43.6	41.5
Mean		27.4	4851	66.6	3594	74.1	780	774	35.1	34.2
StDev		0.7	438.5	0.5	325.1	0.1	42.6	34.4	14.0	13.2
%CV		2.6	9.0	0.8	9.0	0.1	5.5	4.4	39.9	38.6
DONOR3A	4/22/2014	16.5	4000	53.4	2614	65.4	756	751	56.1	54.6
DONOR3B	4/22/2014	15.9	4104	55.0	2656	64.7	616	624	22.0	22.1
DONOR3C	4/22/2014	15.9	4300	56.8	2788	64.8	642	640	21.6	22.6
Mean		16.1	4135	55.1	2686	65.0	671	672	33.2	33.1
StDev		0.3	152.3	1.7	90.8	0.4	74.5	69.2	19.8	18.6
%CV		1.9	3.7	3.1	3.4	0.6	11.1	10.3	59.5	56.2

Appendix Y: Table 6: Assay Transfer, Flow Assay - Continued

Table 6: Assay Transfer	Acquisition Date	MESF tSTAT3 unstim [CD3+ Lymphs]	MESF tSTAT3 unstim [CD3+/CD4+ Lymphs]	%pSTAT3 unstim [CD3+ Lymphs]	%pSTAT3 unstim [CD3+/CD4+ Lymphs]	MESF pSTAT3 unstim [CD3+ Lymphs]	MESF pSTAT3 unstim [CD3+/CD4+ Lymphs]	MESF FMO-PESTIM [CD3+ Lymphs]	MESF FMO-PESTIM [CD3+/CD4+ Lymphs]
DONOR1A	4/22/2014	2014	1982	10.4	11.0	1270	1261	726	729
DONOR1B	4/22/2014	1998	1987	8.8	8.8	1235	1264	692	679
DONOR1C	4/22/2014	1903	1949	9.5	10.4	1287	1395	742	733
Mean		1972	1973	9.6	10.1	1264	1307	720	714
StDev		60.0	20.6	0.8	1.1	26.5	76.5	25.5	30.1
%CV		3.0	1.0	8.3	10.9	2.1	5.9	3.5	4.2
DONOR2A	4/22/2014	1538	1517	10.5	13.6	1150	1221	916	909
DONOR2B	4/22/2014	1960	1930	15.5	19.3	1320	1414	777	772
DONOR2C	4/22/2014	1969	1929	17.6	22.1	1365	1488	842	831
Mean		1822	1792	14.5	18.3	1278	1374	845	837
StDev		246.3	238.2	3.6	4.3	113.4	137.8	69.5	68.7
%CV		13.5	13.3	24.8	23.5	8.9	10.0	8.2	8.2
DONOR3A	4/22/2014	2188	2151	19.0	23.9	1379	1494	655	647
DONOR3B	4/22/2014	1593	1592	10.4	13.2	1104	1174	643	639
DONOR3C	4/22/2014	1604	1606	10.8	13.5	1133	1203	704	688
Mean		1795	1783	13.4	16.9	1205	1290	667	658
StDev		340.4	318.8	4.9	6.1	151.1	177.0	32.3	26.3
%CV		19.0	17.9	36.6	36.1	12.5	13.7	4.8	4.0

Appendix Z: Table 6: Assay Transfer, Flow Assay - Continued

Table 6: Assay Transfer	Acquisition Date	%tSTAT3 STIM [CD3+ Lymphs]	%tSTAT3 STIM [CD3+/CD4+ Lymphs]	MESF tSTAT3 STIM [CD3+ Lymphs]	MESF tSTAT3 STIM [CD3+/CD4+ Lymphs]	%pSTAT3 STIM [CD3+ Lymphs]	%pSTAT3 STIM [CD3+/CD4+ Lymphs]	MESF pSTAT3 STIM [CD3+ Lymphs]	MESF pSTAT3 STIM [CD3+/CD4+ Lymphs]	MFI FMO-PE unstim [CD3+ Lymphs]	MFI FMO-PE unstim [CD3+/CD4+ Lymphs]
DONOR1A	4/22/2014	36.3	34.3	1851	1803	86.5	93.2	12627	13083	368	366
DONOR1B	4/22/2014	31.0	28.2	1766	1708	89.1	95.3	12540	12901	298	299
DONOR1C	4/22/2014	32.5	29.9	1796	1734	88.2	94.3	12038	12309	298	301
Mean		33.3	30.8	1804	1748	87.9	94.3	12402	12764	321	322
StDev		2.7	3.1	43.1	49.1	1.3	1.1	317.9	404.7	40.4	38.1
%CV		8.1	10.1	2.4	2.8	1.5	1.2	2.6	3.2	12.6	11.8
DONOR2A	4/22/2014	50.6	50.4	2093	2080	83.9	97.3	13484	14897	252	253
DONOR2B	4/22/2014	45.2	44.3	1991	1968	83.0	97.5	14121	15651	265	262
DONOR2C	4/22/2014	30.7	30.1	1771	1744	82.6	97.0	13284	14856	281	277
Mean		42.2	41.6	1952	1931	83.2	97.3	13630	15135	266	264
StDev		10.3	10.4	164.6	171.1	0.7	0.3	437.1	447.6	14.5	12.1
%CV		24.4	25.0	8.4	8.9	0.8	0.3	3.2	3.0	5.5	4.6
DONOR3A	4/22/2014	26.8	26.6	1692	1682	85.4	94.0	10881	11883	258	256
DONOR3B	4/22/2014	23.0	23.7	1613	1619	85.6	95.1	11717	12874	210	213
DONOR3C	4/22/2014	27.7	28.1	1699	1693	86.2	95.0	11071	12140	219	218
Mean		25.8	26.1	1668	1665	85.7	94.7	11223	12299	229	229
StDev		2.5	2.2	47.8	39.9	0.4	0.6	438.2	514.3	25.5	23.5
%CV		9.7	8.4	2.9	2.4	0.5	0.6	3.9	4.2	11.1	10.3

Appendix AA: Table 6: Assay Transfer, Flow Assay - Continued

Table 6: Assay Transfer	Acquisition Date	MFI tSTAT3 unstim [CD3+ Lymphs]	MFI tSTAT3 unstim [CD3+/CD4+ Lymphs]	MFI pSTAT3 unstim [CD3+ Lymphs]	MFI pSTAT3 unstim [CD3+/CD4+ Lymphs]	MFI FMO-PE STIM [CD3+ Lymphs]	MFI FMO-PE STIM [CD3+/CD4+ Lymphs]	MFI tSTAT3 STIM [CD3+ Lymphs]	MFI tSTAT3 STIM [CD3+/CD4+ Lymphs]	MFI pSTAT3 STIM [CD3+ Lymphs]	MFI pSTAT3 STIM [CD3+/CD4+ Lymphs]
DONOR1A	4/22/2014	685	674	432	429	248	249	629	613	4262	4415
DONOR1B	4/22/2014	679	676	421	430	236	232	601	581	4233	4354
DONOR1C	4/22/2014	647	663	438	475	253	250	611	590	4064	4155
Mean		670	671	430	445	246	244	614	595	4186	4308
StDev		20.4	7.0	8.6	26.3	8.7	10.1	14.2	16.5	106.9	136.0
%CV		3.0	1.0	2.0	5.9	3.5	4.1	2.3	2.8	2.6	3.2
DONOR2A	4/22/2014	523	516	392	416	312	310	711	707	4550	5025
DONOR2B	4/22/2014	666	656	449	481	265	263	677	669	4764	5278
DONOR2C	4/22/2014	669	656	465	506	287	284	602	593	4483	5011
Mean		619	609	435	468	288	286	663	656	4599	5105
StDev		83.4	80.8	38.4	46.5	23.5	23.5	55.8	58.0	146.8	150.3
%CV		13.5	13.3	8.8	9.9	8.2	8.2	8.4	8.8	3.2	2.9
DONOR3A	4/22/2014	743	731	469	509	224	221	576	572	3675	4012
DONOR3B	4/22/2014	543	538	376	400	220	218	549	551	3956	4345
DONOR3C	4/22/2014	546	546	386	410	240	235	578	576	3739	4098
Mean		611	605	410	440	228	225	568	566	3790	4152
StDev		114.6	109.2	51.1	60.3	10.6	9.1	16.2	13.4	147.3	172.9
%CV		18.8	18.0	12.5	13.7	4.6	4.0	2.9	2.4	3.9	4.2

Appendix BB: Table 7: Assay Transfer, Flow Assay %Stimulation and Acceptance

Table 7: %Stimulation and Assay Transfer Accepance	in CD3+ (Lymphs) Based on %pSTAT	in CD3+/CD4+ (Lymphs) Based on %pSTAT	in CD3+ (Lymphs) Based on MESF pSTAT	in CD3+/CD4+ (Lymphs) Based on MESF pSTAT
AT-Donor 1 (AVG R1-R3)	815.6	833.7	881.0	876.6
AT-Donor 2 (AVG R1-R3)	473.8	431.7	967.0	1001.5
AT-Donor 3 (AVG R1-R3)	539.6	460.4	831.0	853.4
Precision-Donor 1 (AVG R1-R3)	140.7	96.8	720.3	636.9
Precision-Donor 2 (AVG R1-R3)	1430.0	841.9	99.3	855.0
LinDonor 1 @2000 Cells (AVG R1-R3)	2158.3	1483.1	283.9	1241.2
LinDonor 2 @2000 Cells (AVG R1-R3)	80.8	92.1	962.0	888.9
LinDonor 3 @2000 Cells (AVG R1-R3)	74.7	49.2	713.0	635.0
Mean Biological Variability	**714.2**	**536.1**	**682.2**	**873.6**
StDEV Biological Variability	**740.2**	**496.1**	**321.1**	**194.7**
Mean + 1StDEV	**1454.4**	**1032.2**	**1003.3**	**1068.3**
Mean - 1StDEV	**-26.0**	**40.0**	**361.1**	**678.9**

Appendix CC: Gating Strategy