Supplemental Appendix. Non-Hematologic and Hematologic Organ Damage and Additional Disease Features as Secondary Endpoints in Clinical Trials of Advanced SM

Non-Hematologic Organ Damage Not Meeting Eligibility Criteria as a Primary Endpoint

Weight loss

Weight loss is an independent predictor of inferior survival in advanced SM, regardless of whether this results from malabsorption (see manuscript reference 48). Prior response criteria that have used 'normal weight' as a definition of MR, or reversion of weight loss by >50% as the definition of 'GPR' is fraught with challenge (see manuscript references 4 and 24). It is often difficult to identify a patient's 'normal weight'. Weight may also be confounded by variables such as shifts in ascites, use of diuretics, and changes in splenomegaly.

Some protocols have allowed weight loss to be used as a baseline "C" finding if weight loss >10% has occurred in the 6 month period prior to enrollment. However, it is relatively common for patients to lose a substantial portion of weight during an earlier time of their protracted illness, or for weight to demonstrate more recent stability just prior to initiation of new therapy. Formal documentation of pre-trial weight loss is not always feasible and is subject to inaccurate patient recall if medical documentation is unavailable. In addition, improvement of massive ascites with associated weight loss could negatively impact response adjudication. Based on these caveats, we do not believe reverted weight loss is an optimal primary response criterion, especially in the context of clinical trials where an exact quantification of organ damage is mandatory.

Bone Lesions

Similar to ascites or pleural effusions, formal criteria do not exist for evaluating changes in the size of bony lesions. Original response criteria identified "huge" osteolyses and/or severe osteoporosis with pathologic fractures due to local MC infiltration as the basis for evaluating bony disease in advanced SM (see manuscript reference 24). Oral or intravenous bisphosphonates are used for patients with moderate to severe osteoporosis. Cytoreductive agents +/- local irradiation are typically reserved for advanced SM patients with large osteolyses with pathologic fractures.[4] Given the lack of validated quantitative criteria for evaluating changes in lytic lesions and pathologic fractures, and the uncertain nature of what defines 'large osteolyses', we recommend that bone lesions should not be included as evaluable organ damage for response evaluation in clinical trials. Osteoporosis can be evaluated by serial bone mineral density measurements using DEXA scans, but we do not consider it organ damage for the purposes of response adjudication, nor as a defining feature of ASM. New focal pain can be evaluated by skeletal surveys or CT/MRI to evaluate for osteolyses and/or pathologic fractures, and such lesions can be biopsied per the discretion of the investigator to confirm local MC infiltration.

Suggested eligibility and response criteria for weight loss, bone lesions, and <grade 2 non-hematologic organ damage as secondary endpoints are provided in **Supplemental Table 3**.

Hematologic Organ Damage Not Meeting Eligibility Criteria as a Primary Endpoint

Transfusion-Dependent Anemia

We propose that an anemia response in a patient with a baseline transfusion requirement of less than 6 units in the 12 weeks prior to study entry be evaluated as a secondary endpoint. For such patients, response can be described by the % change in transfusions on study during a 12 week period; alternatively, if few transfusions (e.g. <3) are administered in the 12 week pre-treatment period, response based on the absolute increase in hemoglobin (as outlined above) may alternatively be considered. In this regard, it is best to screen for the baseline Hb level immediately before transfusion to ensure that an accurate response is captured.

Transfusion-Dependent Thrombocytopenia

We recommend that baseline platelet transfusion-dependence of less than 6 apheresed units in the 12 weeks prior to study also be evaluated as a secondary endpoint. Similar to anemia, response in such patients can be described by the % change in transfusions during a 12 week on-study period, or based on the absolute increase in the platelet count if few pre-treatment transfusions (e.g. < 3) are administered during the 12 week pre-treatment period.

Additional Disease Features as Secondary Endpoints

KIT Mutation Status

Differences in mutation rates between published cohorts of SM patients may reflect technical disparities in specimen collection/processing and PCR assay sensitivity. Qualitative, semi-quantitative, and quantitative assays for measuring *KIT* D816V allele burden are currently available in clinical and research laboratories, and should be obtained at baseline and at follow-up during evaluation of agents for SM.[1,2] We recommend that PCR with high sensitivity (at least 1%, defined by the specific assay utilized) to detect the *KIT* D816V mutation be performed on the BM. Although it is reasonable to incorporate *KIT* mutation status as a secondary endpoint in clinical trials, the value of reduction of the *KIT* allele burden in the prognosis of treated SM thus far remains unknown. In contrast to the experience with imatinib in chronic myelogenous leukemia, no trials have prospectively validated the utility of molecular remissions of *KIT* in SM. This primarily relates to the fact that only a small number of complete remissions have been recorded in the literature with cytoreductive therapy, therefore currently obviating the need to study the implications of deeper molecular remissions.

Cytogenetic Abnormalities

No recurrent cytogenetic abnormalities have been reported in SM. However, approximately 20% of patients with SM exhibit marrow cytogenetic abnormalities, often associated with the concurrent neoplasm (see manuscript reference 48). Unlike other acute and chronic myeloid disorders, the prognostic significance of karyotype changes in SM has not been

well studied. Often, it cannot be easily elucidated whether cytogenetic abnormalities are related to the SM or myeloid disease (or both), unless assessed in highly-purified compartments of BM cells by FISH and/or PCR-based molecular techniques (see manuscript reference 14). Documentation of partial or complete cytogenetic remissions during therapy is encouraged, but future trials will need to assess the prognostic value of such milestones.

Mediator Symptoms / Quality of Life

Mediator symptoms are prevalent in both indolent and advanced forms of systemic mastocytosis, but do not correlate with MC burden. Cytoreductive therapy can mitigate the frequency and severity of mediator symptoms but may demonstrate no or modest effects on MC burden or organ damage. Symptoms are very heterogeneous and may have a different impact on patients depending on their age, sex, and underlying performance status. In addition, symptom scores can be difficult to apply uniformly and may be altered by a number of protocol and non-protocol drug-related influences.

Changes in disease-related symptoms should be recorded by patients[3] using patient-reported outcomes (PRO) and quality of life (QOL) questionnaires. Examples of PRO and QOL instruments include the 12-item short-form health survey (SF-12), the Memorial Symptom Assessment Scale (MSAS) (see manuscript reference 4), and the MPN-symptom assessment form (MPN-SAF), a validated instrument used to gauge both cytokine-mediated and splenomegaly-related symptoms in myelofibrosis.[5] Although not specifically designed for SM, the MPN-SAF could be modified to specifically capture mediator symptoms such as flushing, diarrhea, skin lesions, and the frequency of anaphylaxis. In addition, psychological testing in

SM patients may be considered by using several tests that evaluate depression, anxiety, and cognitive dysfunction.[6] Tools have also been designed for investigators to grade the severity of patients' constitutional and mediator-related symptoms in CM and SM, as well as response to treatment.[4] Although we recommend that these tools be used in parallel with patient-reported outcomes in study protocols as secondary endpoints, they require further validation in clinical trials of advanced SM patients.

Cutaneous Involvement in Patients with Systemic Mastocytosis

Cutaneous mastocytosis (CM) is a WHO category that is mutually exclusive with SM. Cutaneous involvement is reported at a variable frequency in individuals with ASM, with one series demonstrating that approximately one-third of such patients exhibited no skin lesions.[7] Skin lesions can contribute to impairment of quality of life and burdensome symptoms such as pruritis; however, skin lesions would not typically be an indication for treatment with cytoreductive drugs in advanced SM in the absence of additional findings of organ damage. In older patients, cutaneous lesions may improve without therapy, and evolution to severe disease may be accompanied by a decreasing severity of skin lesions.[8] Photo surveys of CM lesions should be undertaken at baseline and during follow-up of protocol treatment. Standard cytoreductive therapies such as 2-chlorodeoxyadenosine and tyrosine kinase inhibitors (e.g. midostaurin) have demonstrated the ability to induce some regression of skin lesions (see manuscript references 17, 40, 41). However, improvement in CM lesions does not necessarily correlate with higher quality responses such as changes in the burden of SM in the marrow or other extracutaneous organ. Additional tools have been established to grade the extent and

severity of skin lesions and their associated symptoms, and to determine responses to therapy (see manuscript reference 4). We recommend that these tools be incorporated into relevant clinical trials.

Coagulation Abnormalities

Coagulation abnormalities can occur in the presence of advanced SM, usually due to decompensated liver disease, and/or occur more uncommonly due to release of heparin and uncomplexed tPA from mast cells. We encourage documentation of coagulation parameters at baseline as well as descriptive reporting of any responses as part of secondary trial endpoints.

Suggested eligibility and response criteria for secondary endpoints related to these additional disease features are shown in **Supplemental Table 3**.

References for Supplemental Appendix

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3. Hermine O, Lortholary O, Leventhal PS, et al. Case-control cohort study of patients' perceptions of disability in mastocytosis. *PLoS One*. 2008;3(5):e2266.

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Supplemental Table 1. Valent Response Criteria in Aggressive Systemic Mastocytosis and Mast Cell Leukemia

C-Finding	MR (100%)	GPR (>50%)
Bone marrow/blood		
ANC < 1 x 10^9/L	ANC>1 x 10^9/L	Decrease below 1 x 10^9/L reverted by >50%[a]
Anemia, Hb <10 g/dL	Hb>10 g/dL	Decrease below 10 g/dL reverted by >50%[b]
Thrombocytopenia (plt < 100 x 10^9/L)	plt >100 x 10^9/L	Decrease to <100 x 10^9/L reverted by >50% [c]
Liver		
Hepatomegaly w/ascites	No ascites	Decrease of frequency of paracenteses by 50%
Abnormal liver tests		
Elevated enzyme levels	Decrease to normal	Increase reverted by >50%
Hypoalbuminemia	Increase to normal	Decrease reverted by >50%
Portal hypertension	Normal vein pressure	Increase in vein pressure improved by 50%
Spleen		
Palpable splenomegaly with hypersplenism-thrombo-cytopenia	No signs of hypersplenism plt>100 x 10^9/L	Parameters indicating hyperspenism (plt) improved by >50%
GI tract		
Malabsorption with hypoalbuminemia and/or weight loss	Normal albumin Normal weight	Decrease in albumin improved by 50% Weight loss reverted by >50%
Bones		
Huge osteolyses or/and severe osteoporosis w/ pathologic fractures	No osteolyses	Partial resolution of osteolyses,

MR, major response; GPR, good partial response

ANC: absolute neutrophil count; Hb: hemoglobin; plt: platelet count

[a]A minimum increase of 0.1 x 10^9/L ANC is required.

[b]A minimum increase of 1 g/dL Hb is required.

[c]A minimum increase of 10 x 10^9/L platelets is required for GPR.

Supplemental Table 2. Mayo Clinic Revised Response Criteria in Aggressive Systemic Mastocytosis

	Disease-related symptoms[1]	Organomegaly/ Lymphadenopathy[2]	Disease-related Organopathy[3]	Bone marrow (BM) Findings[4]
Response Category	**A**	**B**	**C**	**D**
Complete Response (CR) A+B+C+D required (when present)	Complete resolution for 3 months	Complete resolution[2]	Complete resolution[5]	Absence of abnormal mast cell (MC) infiltration[7]
Major Response (MR) A+B+C+D required (when present)	No progression (at a minimum)	No progression (at a minimum)	Complete resolution of at least one element of Organopathy[3.6]	> 50% decrease in BM MC (%)
Partial Response (PR) A or B or C (without progression in others)	Complete resolution for 3 months	Complete resolution[2]	≥2 grade improvement in at least one element of organopathy[6,8]	No progression (at a minimum)
Stable disease (SD)	None of the above responses			
Progressive Disease (PD) B or C required	Not applicable[9]	>50% increase from baseline	≥2 grade worsening from baseline	Not applicable

Responses are validated only if they last for no fewer than 4 weeks

Correlation between clinical response and change in MC mediator level(s)** and KITD816V allele burden needs further study; recommend prospective sample collection at pre-treatment and at time of peak clinical response for comparison.

**Serum tryptase, 24 -h urine histamine, methylhistamine, and b-Prostaglandin F2a.

1 To be considered as a parameter for response measurement, symptoms must be frequent (occurrence of at least once per month), severe enough to require treatment, despite prophylaxis (H1 and H2 histamine receptor antagonists, proton pump inhibitors, and/or oral cromolyn sodium), and accompanied by either organomegaly/lymphadenopathy or organopathy.

2 Palpable disease or measurable disease by imaging studies required at baseline; baseline and post-treatment status must be documented by imaging studies to allow third-party confirmation of response or progression.

3 ≥Grade 2 ascites (not optimally controlled with medical therapy) OR ≥grade 2 weight loss OR ≥grade 2 osteoporosis (large osteolytic lesions or pathologic fracture) OR ≥Grade 2 anemia (Hgb < 10 g/dL) OR thrombocytopenia (platelet count < 75 X 10^9/L) OR ≥grade 2 hyperbilirubinemia or hypoalbuminemia that is a disease-related change from baseline (grades are per NCI CTC v3.0).

4 BM characteristics to be described: (i) BM MC burden (%) based on tryptase/CD117 (KIT) immunostaining, (ii) cytogenetics, and (iii) *KIT* D816V status.

5 Complete resolution of all evidence of organopathy unless observed changes are deemed related to treatment.

6 No progression in other elements of organopathy should be evident unless observed changes are deemed related to treatment.

7 Cytogenetic remission is not required; cytogenetic response, if any, to be documented as follows: CR disappearance of previously documented chromosomal abnormality without appearance of new ones, and PR at least 50% reduction of cytogenetic abnormality.

8 Per NCI CTC v3.0.

9 Given the difficulty in distinguishing treatment-related symptoms from disease-related symptoms.

Supplemental Table 3. Potential Secondary Eligibility and Response Criteria for Patients not Eligible for CI Determination

Secondary Endpoint	Potential Eligibility Criteria	Potential Response Criteria
Organ Damage without Published Quantification Criteria		
Weight Loss	1) > 10% weight loss in the 6 months prior to treatment start, OR 2) patient or physician documentation of pre-illness weight	1) Reversion of >50% of weight lost in the 6 months prior to treatment; 2) Return to patient's pre-illness weight
Bone Lesions (large osteolyses or pathologic fractures)	Skeletal survey or CT-scan documentation of lytic lesions and/or pathologic fracture(s)	> 50% decrease in the number of bone lesions vs. pre-trial
Portal hypertension	Abnormal portal vein flow	Normalization of portal vein flow
Organ Damage Not Eligible for CI Response		
Non-Hematologic		
Ascites/pleural effusion	Less than grade 2 ascites or pleural effusion (e.g. asymptomatic and not requiring intervention OR symptomatic (grade 2) and only requiring 1 paracentesis or thoracentesis in prior 12 weeks	No ascites or pleural effusion AND no need for paracentesis or thoracentesis
Liver dysfunction	Less than grade 2 and at least 1.5x ULN of AST, ALT, direct bilirubin, or AP[a] in the presence of ascites and/or portal hypertension, OR ≥ grade 2 AST, ALT, direct bilirubin, or AP[a] in the absence of ascites and/or portal hypertension	Normalization of one or more liver function test(s)
Hypoalbuminemia	Less than grade 2 hypoalbuminemia	Normalization of albumin and at least 0.5 g/dL increase
Hematologic		
Neutropenia	ANC ≥1 x 10^9/L but less than LLN	Normalization of the ANC and an absolute increase of ≥0.5 x 10^9/L
Anemia (TI)	Grade 1 anemia (<LLN – Hb 10 g/dL)	Hb increase of ≥1.5 g/dL (may also be used as a secondary response endpoint to capture patients with >= Grade 2 anemia at baseline, but who do not achieve a >= 2 g/dL Hb increase)
Anemia (TD)	<6 transfusions in the 12-week pre-treatment period given for a Hb ≤ 8.5 g/dL, including at least 1 unit in the last 28 days	% change in number of transfusions or absolute increase in Hb
Thrombocytopenia (TI)	Grade 1 thrombocytopenia	Normalization of the platelet count and absolute increase of ≥50,000/mm^3
Thrombocytopenia (TD)	<6 transfusions in the 12-week pre-treatment period for a platelet count <20,000/mm^3, including at least 1 unit in the last 28 days	% change in number of transfusions or absolute increase in platelet count
Additional Secondary Endpoints		
KIT mutation status	Qualitative or (semi)-quantitative RT-PCR for *KIT* D816V or other activating *KIT* mutation in bone marrow or blood	>50% decrease in KIT allele burden from similar tissue compartment (e.g. bone marrow or blood) by semi-quantitative or quantitative RT-PCR with at least 1% sensitivity
Cytogenetic Abnormalities	SM- or myeloid neoplasm-associated chromosome abnormalities	Complete, partial, or minor cytogenetic response
Mediator symptoms/ QOL	Pre-treatment survey of QOL and symptoms	Examples include SF-12, MSAS, modification of MPN-SAF, investigator grading of mediator symptoms
Cutaneous mastocytosis (CM)	Histological /immunohistochemical confirmation of CM; photo survey of CM lesions	Reduction in number and/or intensity of CM lesions
Coagulation abnormalities	SM-related abnormalities of PT/INR, PTT; increased heparin level	Normalization of PT/INR and PTT; heparin level not increased

[a] GGT can be used to determine the liver vs. bone origin of alkaline phosphatase but is not considered eligible as a liver-related organ damage laboratory abnormality

CI: Clinical improvement; CM: cutaneous mastocytosis; ; QOL: quality of life; LLN: lower limit of normal; TI: transfusion-independent; TD: transfusion-dependent; RT-PCR: reverse-transcriptase polymerase chain reaction; SF-12: 12-item short form health survey: MSAS: Memorial Symptom Assessment Scale; MPN-SAF: Myeloproliferative Neoplasm Symptom Assessment Form.