Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

Supplement to: Chari A, Vogl DT, Gavriatopoulou M, et al. Oral selinexor–dexamethasone for triple-class refractory multiple myeloma. N Engl J Med 2019;381:727-38. DOI: 10.1056/NEJMoa1903455

Supplementary Material

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Efficacy Endpoint Definitions

Overall Response Rate (ORR): included patients who experienced partial response (PR), very good partial response (VGPR), CR, or sCR, based on IMWG response criteria. The ORR for patients with penta-exposed, triple-class-refractory MM in Part 2 was compared to a minimal threshold level of 10%.

Duration of response (DOR): for patients with a confirmed response, the duration from first response (at least PR) to time of progressive disease (PD; per IRC) or death due to PD, whichever occurred first.

Clinical Benefit Rate (CBR): proportion of patients who achieved a confirmed MR or better.

Progression-free Survival (PFS): duration from start of study treatment to Time of PD (per IRC) or death from any cause, whichever occurred first.

Overall Survival (OS): duration from start of study treatment to death from any cause. If a death event did not occur during the follow-up period, the patient was censored at the date of discontinuation from the study, censored at the date of discontinuation from the study, or date of last participating visit (e.g., a telephone contact with patient status being alive), or database cut date, whichever is earlier.

Minimal Response (MR): ≥ 25% but < 49% reduction of serum M protein and reduction in 24 h urine M protein by 50–89%, which still exceeded 200 mg per 24 h. In addition to the above criteria, if present at baseline, 25–49% reduction in the size of soft tissue plasmacytomas was also required. No increase in size or number of lytic bone lesions (development of compression fracture does not exclude response).

Revised International Myeloma Working Group (IMWG) Diagnostic Criteria for Multiple Myeloma

Clonal bone marrow plasma cells ≥10% or biopsy-proven bony or extramedullary plasmacytoma* and any one or more of the following myeloma-defining events:

- Evidence of end organ damage that can be attributed to the underlying plasma cell proliferative disorder, specifically:
    - Hypercalcemia: serum calcium >0.25 mmol/L (>1 mg/dL) higher than the upper limit of normal or >2·75 mmol/L (>11 mg/dL)
    - Renal insufficiency: creatinine clearance <40 mL per min† or serum creatinine >177 µmol/L (>2 mg/dL)
    - Anemia: hemoglobin value of >20 g/L below the lower limit of normal, or a hemoglobin value <100 g/L
    - Bone lesions: one or more osteolytic lesions on skeletal radiography, CT, or PET-CT‡
- Any one or more of the following biomarkers of malignancy:
- Clonal bone marrow plasma cell percentage* ≥60%
- Involved:uninvolved serum free light chain ratio§ ≥100
- • >1 focal lesion(s) on MRI studies¶

*Clonality should be established by showing κ/λ-light-chain restriction on fl ow cytometry, immunohistochemistry, or immunofluorescence. Bone marrow plasma cell percentage should preferably be estimated from a core biopsy specimen; in case of a disparity between the aspirate and core biopsy, the highest value should be used. †Measured or estimated by validated equations. ‡If bone marrow has less than 10% clonal plasma cells, more than one bone lesion is required to distinguish from solitary plasmacytoma with minimal marrow involvement. §These values are based on the serum Freelite assay (The Binding Site Group, Birmingham, UK). The involved free light chain must be ≥100 mg/L. ¶Each focal lesion must be 5 mm or more in size.

Quality of Life (QoL)

Methods

Quality of life and potential for improvement over the course of the study was assessed using the Functional Assessment of Cancer Therapy–Multiple Myeloma (FACT-MM) patient-reported outcome questionnaire. This instrument combines the general version of the FACT (FACT-G) with a MM-specific subscale (14 items). The subscales for the FACT-G are Physical Well-Being (7 items), Social/Family Well-Being (7 items), Emotional Well-Being (6 items), and Functional Well-Being (7 items). The trial outcomes index (TOI; total of 41 items) was the primary measurement of interest, comprised of the Physical and Functional subscales plus the MM-specific subscale. Each item is rated on a 5-point Likert scale, ranging from 0 ("Not at all") to 4 ("Very much"); therefore, the TOI has a score ranging from 0 to 120.

The QoL assessment was performed at Baseline (prior to first dose of study treatment), Day 1 of each cycle on or after the second cycle, and at the Final visit. The primary analysis for QoL was based on the change from baseline on the TOI score at each assessment time point, which was summarized using descriptive statistics including mean, standard deviation, median, minimum, and maximum. The total score considering all 5 subscales as well as the 5 individual subscale sums of scores was summarized similarly.

Results

FACT-MM Scales	Median (range) Baseline Score n=107	Median (range) Change from Baseline at Day 1 of Cycle					
		Cycle 2 n=76	Cycle 3 n=50	Cycle 4 n=31	Cycle 5 n=26	Cycle 6 n=19	Cycle 7 n=12
Total Score[a]	107 (54, 158)	–6 (–69, 54)	–12 (–54, 40)	–7 (–69, 43)	–11 (–31, 27)	–17 (–41, 25)	–14 (–37, 17)
Trial Outcome Index[b]	67 (28, 108)	–4 (–61, 43)	–8 (–44, 23)	–7 (–53, 25)	–9 (–27, 17)	–10 (–39, 11)	–13 (–33, 11)
PWB Subscale	20 (5, 28)	–3 (–23, 12)	–4 (–21, 7)	–4 (–17, 10)	–4 (–17, 11)	–3 (–17, 3)	–6 (–20, 1)
SWB Subscale	24 (8, 28)	0 (–13, 15)	–1 (–13, 9)	–1 (–9, 13)	0 (–9, 7)	0 (–9, 7)	–1 (–9, 4)
EWB Subscale	17 (4, 24)	0 (–7, 11)	1 (–11, 12)	1 (–9, 12)	0 (–7, 10)	2 (–6, 11)	1 (–4, 6)
FWB Subscale	15 (1, 28)	–2 (–18, 14)	–2 (–16, 7)	–1 (–19, 7)	–1 (–16, 10)	0 (–11, 6)	–2 (–11, 9)
MMS Subscale	33 (13, 54)	–2 (–23, 30)	0 (–26, 21)	0 (–32, 24)	–1 (–15, 11)	0 (–16, 10)	–5 (–15, 10)

[a]Total Score=PWB+SWB+EWB+FWB+MMS

[b]Trial Outcome Index=PWB+FWB+MMS

PWB=Physical Well-Being subscale, SWB=Social/Family Well-Being subscale, EWB=Emotional Well-Being subscale, FWB=Functional Well-Being subscale, MMS=Multiple Myeloma subscale.

Table S1. Inclusion/Exclusion Criteria

Inclusion Criteria	Exclusion Criteria
Measurable disease based on IMWG guidelines as defined by at least 1 of the following: • Serum M-protein ≥0.5 g/dL by SPEP or quantitative IgA • Urinary M-protein excretion ≥200 mg/24 hours • FLC ≥100 mg/L, provided that the FLC ratio is abnormal Previously received ≥3 anti-MM regimens including: an alkylating agent, lenalidomide, pomalidomide, bortezomib, carfilzomib, daratumumab, and a glucocorticoid Refractory to previous anticancer treatments: glucocorticoids, proteasome inhibitor (i.e. bortezomib and/or carfilzomib), IMiD (i.e. lenalidomide and/or pomalidomide), and daratumumab − Refractory: ≤25% response to therapy or progression during or within 60 days after completion of therapy Refractory to most recent anti-MM regimen Adequate hepatic function • Total bilirubin <2 × ULN (Gilbert's syndrome: <3 × ULN) • AST <2.5 × ULN • ALT <2.5 × ULN Adequate renal function • Estimated creatinine clearance of ≥20 mL/min per Cockcroft/Gault Eastern Cooperative Oncology Group (ECOG) performance status (PS) ≤2 Adequate hematopoietic function • Total WBC count >1000/mm^3 • ANC ≥1000/mm^3 • Platelet count ≥75,000/mm^3 for patients with <50% of bone marrow nucleated cells are plasma cells; or ≥50,000/mm^3 for patients with ≥50% of bone marrow nucleated cells are plasma cells • Hemoglobin ≥8.5 g/dL (>8.0 g/dL with approval from medical monitor)	Active smoldering MM Active plasma cell leukemia Documented systemic amyloid light chain amyloidosis Active CNS MM Active GVHD (after allogeneic stem cell transplantation) on day 1 of cycle 1 Life expectancy of <4 months Active, unstable cardiovascular function HIV seropositive Known active hepatitis A, B, or C infection; or known to be positive for HCV RNA or HBsAg (HBV surface antigen) Prior malignancy that required treatment or has shown evidence of recurrence (except for non-melanoma skin cancer or adequately treated cervical carcinoma *in situ*) within 5 years prior to enrollment Grade ≥3 peripheral neuropathy or Grade ≥2 painful neuropathy Participation in an investigational anticancer study within 21 days prior to cycle 1 day 1 Receipt of transfusions as follows: • Platelet infusion within 1 week prior to cycle 1 day 1 • RBC transfusion within 2 weeks prior to cycle 1 day 1 Receipt of the following blood growth factors within 2 weeks prior to cycle 1 day 1: G-CSF, GM-CSF, EPO, or megakaryocyte growth factor Prior exposure to a SINE compound, including selinexor

CNS=central nervous system, G-CSF=granulocyte colony stimulating factor, GM-CSF=granulocyte-macrophage colony stimulating factor, EPO=erythropoietin, GVHD=graft versus host disease, HBsAg=hepatitis B virus surface antigen, HCV=hepatitis C virus, HIV=human immunodeficiency virus, MM=multiple myeloma, RNA=ribonucleic acid.

Table S2. Selinexor-related Toxicities Leading to Dose Modification (See Supplementary Table S3 for dose levels)

Toxicity	**Dose Modification**
Fatigue (Grade 2 for >7 days or Grade 3)	Interrupt selinexor until return to Grade 1 or baseline; when resolved: 1st occurrence: restart selinexor at current dose 2nd occurrence: reduce selinexor by 1 dose level
Anorexia (≥Grade 2) or Weight Loss (≥Grade 3)	Interrupt selinexor until return to Grade 1 or baseline; when resolved: 1st occurrence: reduce selinexor by 1 dose level 2nd occurrence: discuss with Sponsor's Medical Monitor
Nausea (Grade 3)	Interrupt selinexor until return to Grade 2 or baseline; when resolved, reduce selinexor by 1 dose level
Hyponatremia (persistent or symptomatic Grade 3 or Grade 4)	Interrupt selinexor until return to Grade 1 or baseline; when resolved, reduce selinexor by 1 dose level
Diarrhea (Grade 2)	Interrupt selinexor until return to Grade 1 or baseline; when resolved 1st occurrence: restart selinexor at current dose 2nd occurrence: reduce selinexor by 1 dose level
Diarrhea (≥Grade 3)	Interrupt selinexor until return to Grade 1 or baseline; when resolved, reduce selinexor by 1 dose level
Thrombocytopenia (Grade 3 without bleeding)	<u>Patients at Dose Level 0</u> 1st occurrence: Continue dosing and reduce to Dose Level -2 once weekly until recovery to Grade 2 or baseline; thereafter may resume at Dose Level -2 twice-weekly. <u>Patients at other Dose Levels</u> 1st occurrence: Reduce by 1 dose level once weekly until recovery to Grade 2 or baseline; thereafter may resume dose on twice-weekly schedule. 2nd occurrence: Hold selinexor until recovery to Grade 2 or baseline; when resolved, resume at 1 dose level lower QW
Thrombocytopenia (Grade 4 without bleeding)	Interrupt selinexor until return to Grade 3 or baseline; when resolved: <u>Patients at Dose Level 0</u> 1st occurrence: reduce to Dose Level -2 once weekly until recovery to Grade 2 or baseline; thereafter may resume at Dose Level -2 twice-weekly. <u>Patients at other Dose Levels</u> 1st occurrence: Reduce by 1 dose level once weekly until recovery to Grade 2 or baseline; thereafter may resume dose on twice-weekly schedule.

	2nd occurrence: Hold selinexor until recovery to Grade 2 or baseline; when resolved, resume at 1 dose level lower QW
Thrombocytopenia (Grade 3 with bleeding)	Interrupt dosing until bleeding has stopped and the patient is clinically stable. Restart upon recovery to ≤Grade 2 or baseline; once resolved: <u>Patients at Dose Level 0</u> 1st occurrence: reduce to Dose Level -2 once weekly until recovery to Grade 1 or baseline; thereafter may resume at Dose Level -2 twice-weekly. <u>Patients at other Dose Levels</u> 1st occurrence: reduce by 1 dose level once weekly until recovery to Grade 1 or baseline; thereafter may resume dose on twice- weekly schedule.
Neutropenia (≥Grade 3, with or without fever)	Interrupt dosing until recovery to Grade 2 or baseline and without fever (if febrile) and the patient is clinically stable. Reduce selinexor by 1 dose level when resuming.
All other Selinexor-related AEs (≥Grade 3)	Interrupt selinexor until recovery to ≤Grade 2 or Baseline; when resolved, reduce selinexor by 1 dose level.

Grade is based on CTCAE criteria. QW = once weekly.

Table S3. Pre-specified Dose/Schedule Modifications for Adverse Events Related to Study Drug

	Dose Level	**Selinexor Dosing**
Dose Increase	1	100 mg twice-weekly (200 mg total per week)
Starting Dose	0	80 mg twice-weekly (160 mg total per week)
Dose Reduction	–1	60 mg twice-weekly (120 mg total per week)
	–2	100 mg total per week: 100 mg once weekly *OR* divided as 60 mg and 40 mg on separate days
	–3	80 mg total per week: 80 mg once weekly *OR* divided as 40 mg separate days
	–4	60 mg total per week: 60 mg once weekly *OR* divided as 40 mg and 20 mg on separate days
	–5	40 mg total per week: 40 mg once weekly *OR* divided as 20 mg on separate days

Table S4. Baseline Demographic and Disease Characteristics in the Modified Intent-to-treat Population[a]

Characteristic	N=122
Age	
Median (range) — yr	65.2 (40–86)
Distribution — no. (%)	
18–50	8 (6.6)
51–64	52 (42.6)
65–75	44 (36.1)
>75	18 (14.8)
Sex — no. (%)	
Male	71 (58.2)
ECOG PS — no. (%)	
0	36 (29.5)
1	71 (58.2)
2	11 (9.0)
Missing	4 (3.3)
Creatinine Clearance — no. (%), mL per minute	
<40	14 (11.5)
40 to <60	25 (20.5)
≥60	82 (67.2)
Missing	1 (<0.1)
Neutropenia[b] — no. (%)	
Grade 1	11 (8.6)
Grade 2	8 (6.5)
Grade 3	1 (0.8)
Grade 4	0
Thrombocytopenia[b] – no. (%)	
Grade 1	34 (27.6)
Grade 2	9 (7.3)
Grade 3	1 (0.8)
Grade 4	0
Revised ISS Stage — no. (%)	
I	20 (16.4)
II	78 (63.9)
III	23 (18.9)
Unknown	1 (0.8)
Bone Marrow Plasma Cells — (%)	
Mean (SD)	27.0 (30.4)
Chromosomal Abnormality — no. (%)	
High risk overall[c]	65 (53.3)
del(17p)/p53	32 (26.2)
t(4;14)	17 (13.9)
t(14;16)	5 (4.1)
gain(1q)	40 (32.8)
Time Since Initial Diagnosis	
Median (range) —yr	6.6 (1.1–23.4)
Prior Treatment Regimens	
Median (range) — no.	7 (3–18)
Time Since Discontinuation of Last Treatment	
Median (range) — weeks	4.1 (0.1–26.0)

Refractoriness to Select Prior Therapies — no. (%)[d]	
At least 1 IMiD, 1 PI, and daratumumab	122 (100)
Carfilzomib, pomalidomide, and daratumumab	117 (95.9)
Carfilzomib, lenalidomide, pomalidomide, and daratumumab	101 (82.8)
Bortezomib, carfilzomib, pomalidomide, and daratumumab	94 (77.0)
Bortezomib, carfilzomib, lenalidomide, pomalidomide, and daratumumab (Penta-refractory)	83 (68.0)

[a]123 patients were enrolled; 1 patient did not meet eligibility criteria resulting in 122 patients included in the modified intent-to-treat population.
[b]Graded by Clinical Terminology Categories for Adverse Events (CTCAE) v4.03.[20] Grade 1 = Mild. Grade 2 = Moderate. Grade 3 = Severe. Grade 4 = Life Threatening.
[c]Includes any of del(17p)/p53, t(14; 16), t(4; 14), or 1q21 (1q gain >2).
[d]Defined as refractory to an anti-myeloma therapy if a) best response on the therapy is SD or worse, or b) patient progressed or relapsed either during treatment or within 60 days after discontinuing the therapy.

Table S5. Adverse Events Leading to Study Drug Discontinuation With ≥1 Event Related to Selinexor or Dexamethasone

Adverse Event	**N=123**
Patient with ≥ 1 TEAE, n (%)	40 (32.5)
Related[a]	22 (17.9)
Not Related	17 (13.8)
Adverse Events — % related/% unrelated	
Nausea	5.7/0
Fatigue	4.9/0
Decreased weight	4.1/0
Asthenia	3.3/0.8
Thrombocytopenia	2.4/0.8
Pneumonia	2.4/0.8
Vomiting	2.4/0
Decreased appetite	1.6/0
General physical health deterioration	0.8/0.8
Diarrhea	0.8/0
Confusional state	0.8/0
Cognitive disorder	0.8/0
Dehydration	0.8/0
Dizziness	0.8/0
Fungal infection	0.8/0
Renal failure	0.8/0

[a]Possible, probable, or definite relation to study drug as assessed by the investigator.

Table S6. Serious Adverse Events Occurring in ≥2% Patients

Adverse Event — n (%)	N=123	
	Treatment-emergent	Treatment-related[b]
Patient with ≥ 1 serious treatment emergent	78 (63.4)	39 (31.7)
Pneumonia	14 (11.4)	6 (4.9)
Sepsis	11 (8.9)	2 (1.6)
Anemia	4 (3.3)	1 (0.8)
Fatigue	4 (3.3)	3 (2.4)
General physical health deterioration	4 (3.3)	2 (1.6)
Hyponatremia	4 (3.3)	3 (2.4)
Mental status changes	4 (3.3)	1 (0.8)
Bacteremia	4 (3.3)	1 (0.8)
Asthenia	3 (2.4)	3 (2.4)
Pyrexia	3 (2.4)	0
Dehydration	3 (2.4)	3 (2.4)
Confusional state	3 (2.4)	1 (0.8)
Acute kidney injury	3 (2.4)	3 (2.4)

[a]Based on CTCAE v4.03

[b]Subset of treatment-emergent events. Relatedness per investigator assessment.

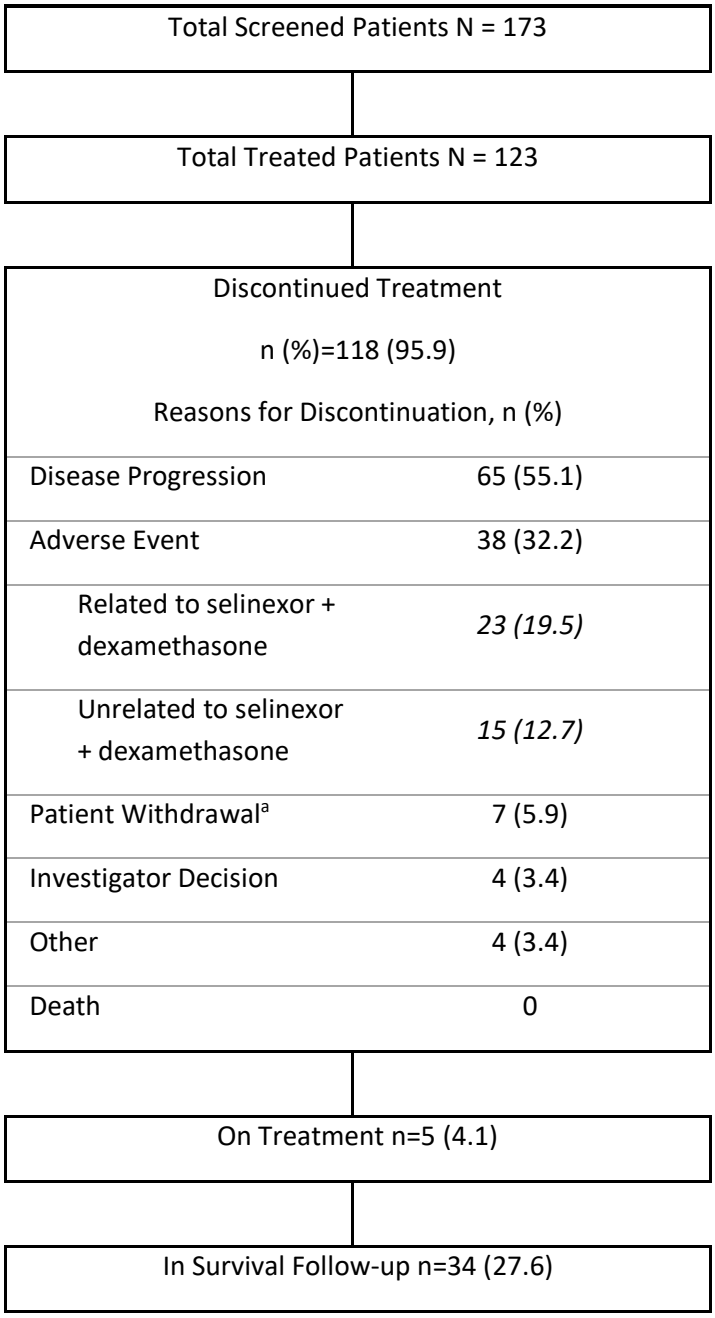
Table S7. Post-study Drug Anti-Myeloma Therapy

Therapy	n = 61
Proteasome inhibitor, immunomodulatory drug, daratumumab ± chemotherapy	43
Stem cell transplant	10
Immunotherapy	9
Venetoclax-containing regimen	9
Panobinostat-containing regimen	7
CAR-T	3

Table S8. Response by FISH

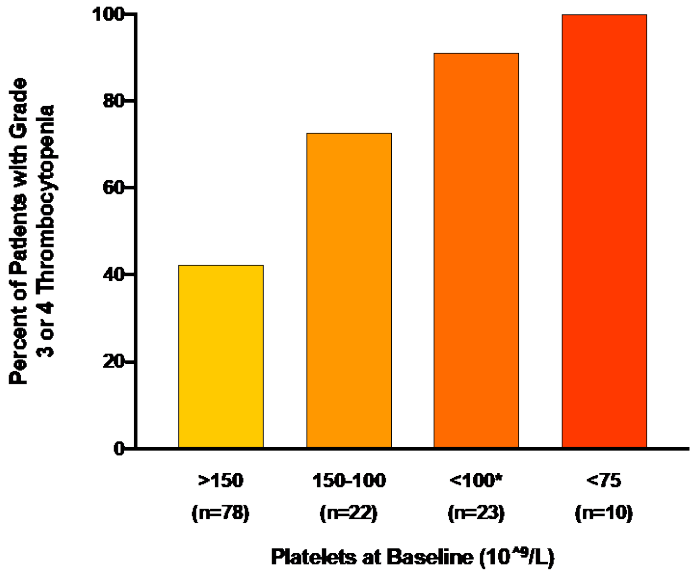
FISH	Response Rate, n (%); 95%CI
del (17p)/p53 (n = 32)	4 (12.5); 95%CI, 3.5, 29
t(4,14) (n = 17)	4 (23.5); 95% CI, 6.8, 49.9
t(14,16) (n = 5)	1 (20); 95% CI, 0.5, 71.6
Gain 1q (n = 40)	8 (20); 95% CI, 9.1, 35.6

Figure S1. Patient Disposition



[a]Includes 3 patients lost to follow-up.

Figure S2. Relationship between the incidence of grade[a] 3 or 4 thrombocytopenia and baseline platelet count



[a]Graded by CTCAE v4.03

Pharmacodynamics

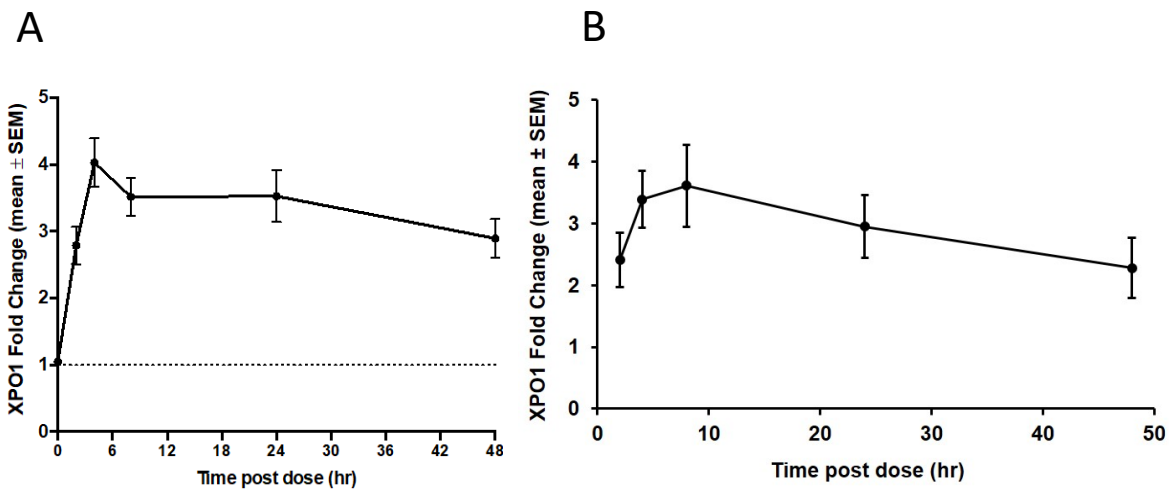
XPO1 mRNA induction as a pharmacodynamic marker in selinexor-treated patients

Selinexor binding to XPO1, results in immediate inactivation of nuclear export and leads to XPO1 protein degradation [Tai, 2014]. In parallel, induction of XPO1 mRNA transcription that does not result with newly synthesized protein was observed in normal and cancer cells. We used the XPO1 mRNA induction as a pharmacodynamic marker in selinexor treated patients [Abdul-Razak 2016; Alexander 2016].

Methods: In a Phase 1 study of selinexor for the treatment of MM, acute myelogenous leukemia (AML), or Non-Hodgkin lymphoma (NHL), peripheral blood was collected from 36 patients (14 of whom had MM). Patients received oral selinexor ranging from 3 to 40 mg/m^2 [Chen 2017]. Samples were collected predose and at prespecified timepoints (ranging from 0.5 to 48 hours post administration of selinexor). The XPO1 mRNA in peripheral blood cells was measured using quantitative polymerase chain reaction (qPCR). Statistical comparisons were performed using analysis of variance (ANOVA) and Dunnets's post-hoc test.

Results: In patients with MM, AML, or NHL, the F_{max} in XPO1 mRNA occurred at approximately 4 hours following administration of selinexor (Figure S3A). The increases observed at 2 hours or later were significantly higher than predose levels ($P \leq 0.0001$). The increase in XPO1 mRNA continued to be elevated for 48 hours. Of note, the terminal half-life of selinexor in plasma is 5 to 8 hours. Therefore, the induction of XPO1 mRNA levels is substantially longer than the half-life of selinexor in plasma [Gounder 2016; Abdul Razak 2016]. Similar results were observed in the 14 patients with MM (Figure S3B).

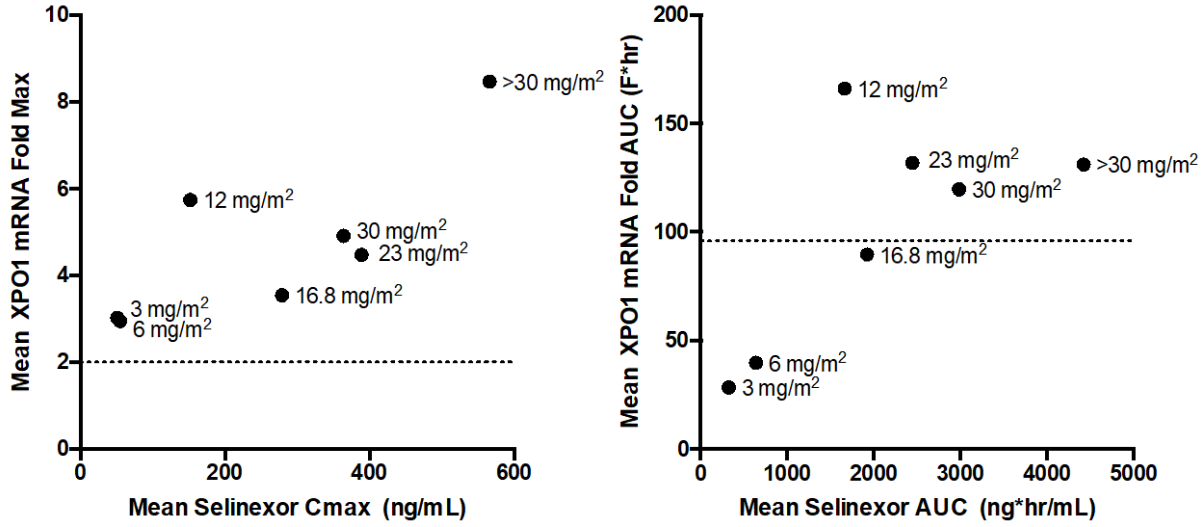
Figure S3. Mean induction of XPO1 expression in patients with a hematological malignancy following a single, oral dose of selinexor (3 to 40 mg/m^2). Panel A depicts all 36 patients who had either multiple myeloma, acute myelogenous leukemia, or non Hodgkins Lymphoma. Panel B depicts the 14 patients with multiple myeloma [Chen 2017]



Across the different doses tested (3-40 mg/m^2) in patients with MM, AML, or NHL, the XPO1 mRNA induction was >2-fold for all dose levels and generally increased with selinexor dose and exposure reaching maximal induction at doses ≥12 mg/m^2 (~30 mg) with mean AUC exposures of ≥1662 ng·hr/mL (Figure S4).

In general, the F_{max} was achieved in 4 to 8 hours post dose and coincided with the C_{max} for most doses of selinexor. A significant, positive correlation (r^2=0.7738; p=0.0412) was observed between mean XPO1 mRNA F_{max} and plasma C_{max} of selinexor (Figure S4). A positive correlation was also observed between mean XPO1 mRNA F_{max}· fold AUC and selinexor plasma AUC; however, this correlation only trended towards significance (r^2=0.6737; p=0.0971).

Figure S4. Correlation of selinexor plasma exposure and XPO1 mRNA induction.



Conclusions: XPO1 mRNA was induced in a dose-dependent manner with levels significantly correlated with the overall pharmacokinetic profile of selinexor. The peak of XPO1 mRNA induction (F_{max}) for all doses was ~4 hours and XPO1 mRNA levels remained elevated for 48 hours after administration of selinexor. There was no difference in the extent of XPO1 induction when evaluated by tumor type. The half-life of the XPO1 mRNA induction exceeded that of selinexor plasma concentration suggesting a prolonged pharmacodynamic effect. This difference may be related to both the covalent mechanism of drug action and the relatively long half-life of the XPO1 protein >24 hrs.

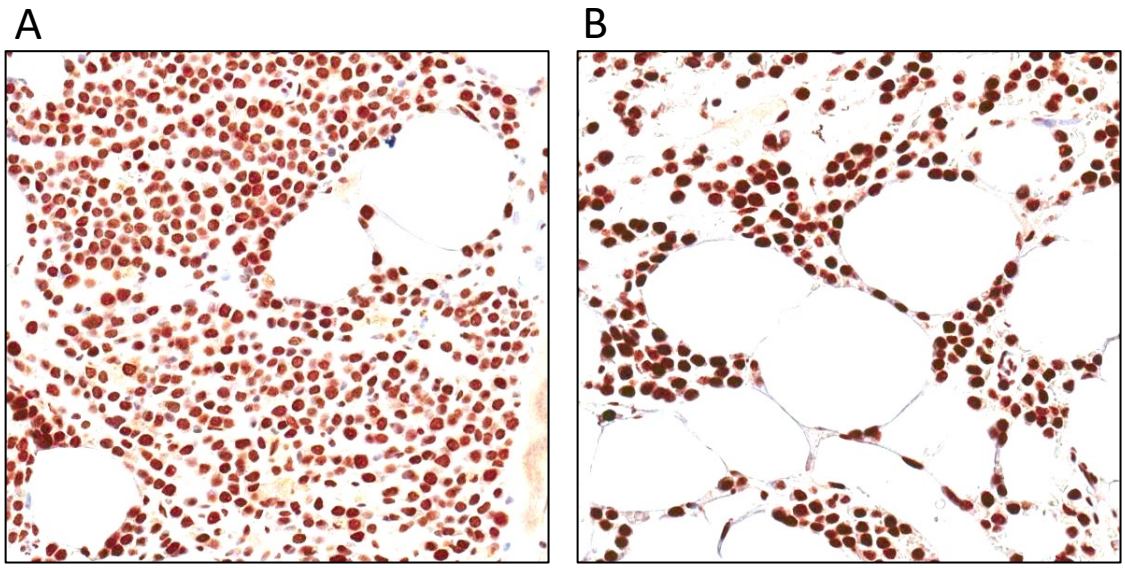
Selinexor Synergizes with Dexamethasone, Induces Nuclear GR Expression in R/R Multiple Myeloma Human Biopsy

Selinexor synergizes with dexamethasone to repress the mTORC1 signaling pathway and to induce MM cell death [Argueta 2018]. Drug combination induces the expression of the glucocorticoid receptor expression in multiple myeloma (MM) patients.

Methods: Bone marrow trephine biopsy samples were collected from a patient with relapsed/refractory MM at 2 time points (predose and on Day 10 of Cycle 2) during treatment with selinexor 80 mg plus dexamethasone 20 mg, twice weekly. This patient achieved a best response of PR while on selinexor. Expression of the glucocorticoid receptor was detected with immunohistochemistry using a monoclonal antibody specific for the glucocorticoid receptor and 3,3'-diaminobenidine (DAB) as the detection substrate (glucocorticoid receptor stains dark brown).

Results: Representative staining of predose (Figure S5A) and Cycle 2 Day 10 (Figure S5B) bone marrow samples indicate an increased nuclear staining of the glucocorticoid receptor on Cycle 2 Day 10 compared with predose.

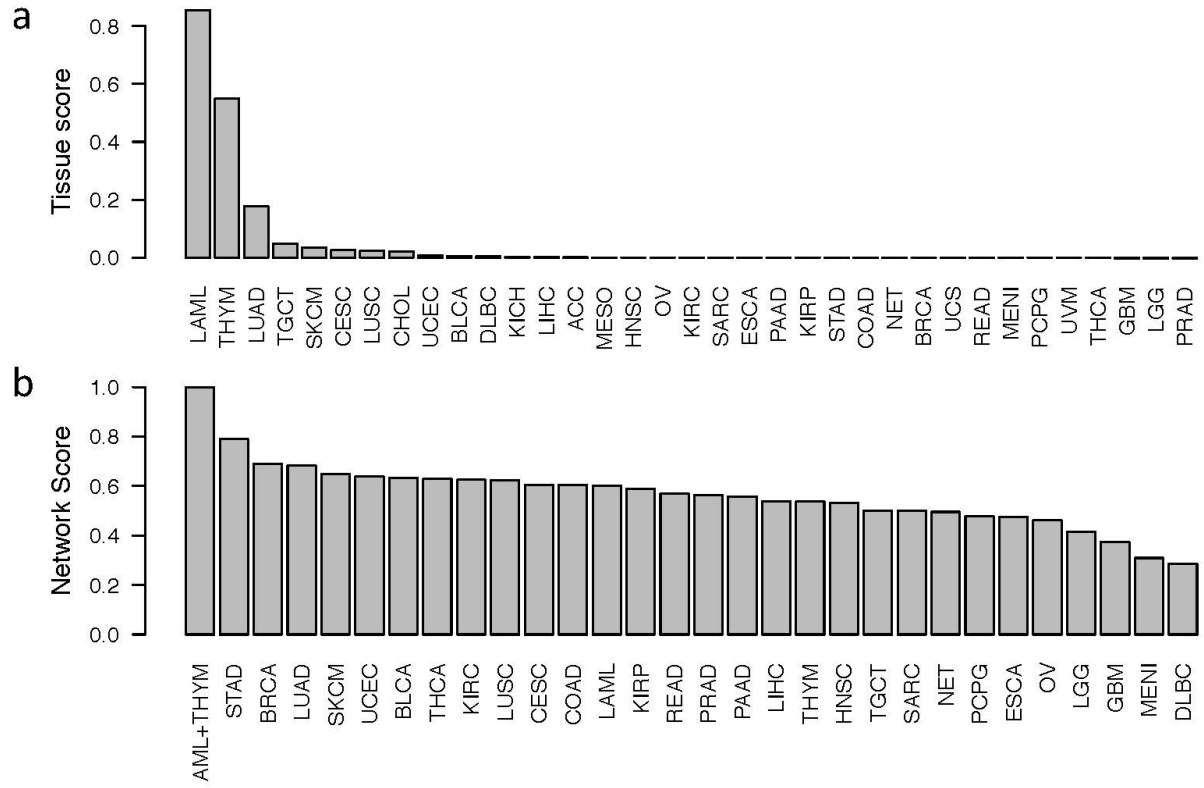
Figure S5. Selinexor synergizes with dexamethasone, induces nuclear GR expression in R/R multiple myeloma human biopsy



Biomarkers of selinexor response in MM

Methods: Pre-treatment bone marrow biopsy samples were collected per protocol in a pre-specified fashion with the intention of identifying predictive markers of response. The specific analyses performed here were carried out on a post-hoc basis as the methods had not been chosen on protocol initiation and the patients' responses were known at the time of the analyses. The transcriptome for 2 separate batches of pre-treatment biopsies, from patients enrolled in the STORM (Parts 1 and 2) trial, was profiled by RNA-Seq. The activity of 6,204 regulatory proteins was inferred by metaVIPER [Ding 2018], using acute myeloid leukemia (AML) and thymoma context-specific model of transcriptional regulation (interactomes), which were selected among 29 available interactomes based on tissue lineage supervised classification and network representation analysis (Supplementary Figure S6)[Alvarez 2016]. Unlike raw gene expression profiles, VIPER-inferred protein activity is extremely reproducible, and this methodology (DarwinOncoTarget algorithm) has been approved by the NYS Department of Health CLIA/CLEP Validation Unit for Molecular and Cellular Tumor Markers for Oncology [Neal 2019].

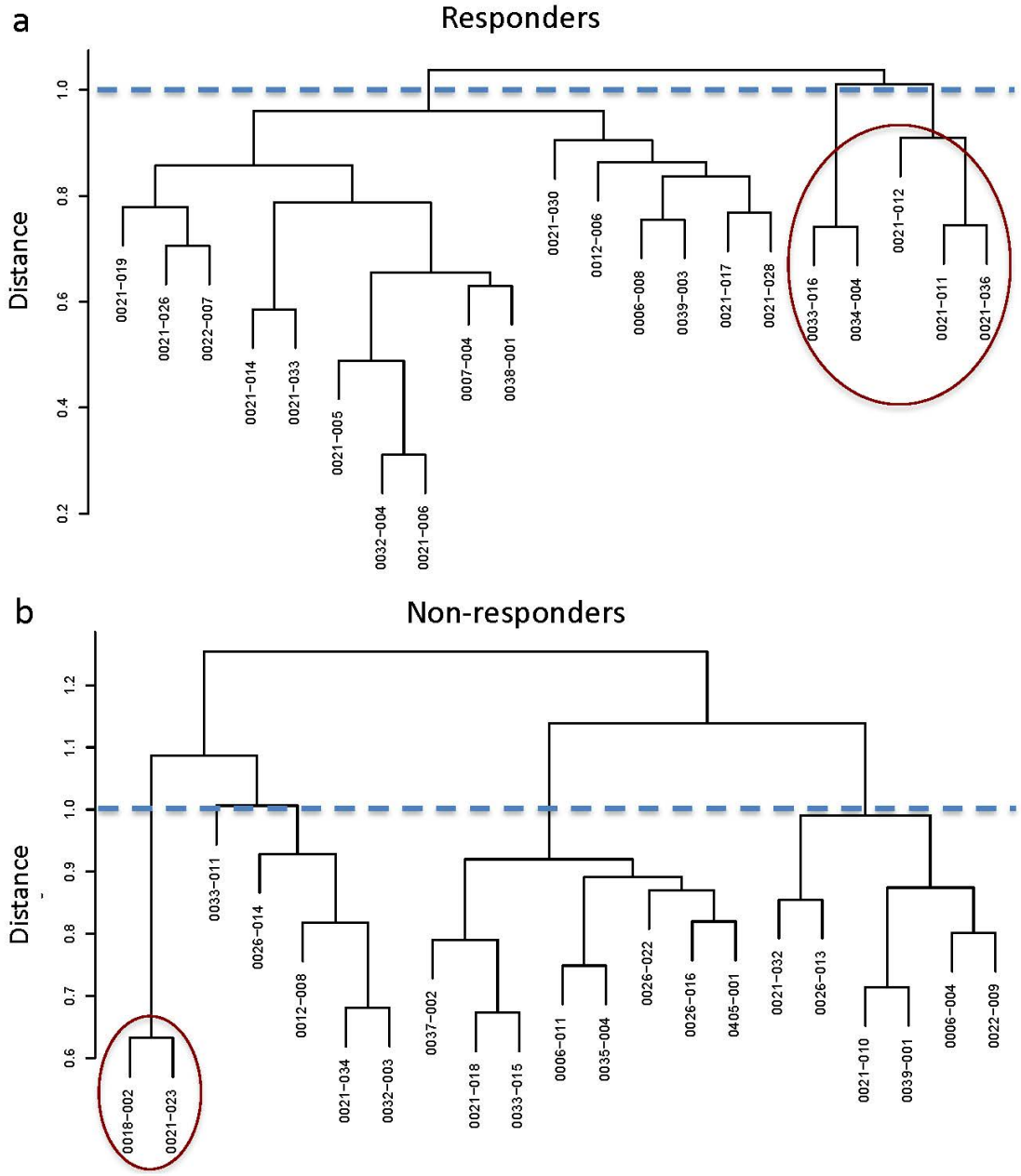
Figure S6. Identification of the most appropriate tissue context-specific interactomes for MM based on the likelihood predicted by a tissue-type classifier based on gene expression (a), and the Network Score, representing how well each evaluated interactome can explain the transcriptional state of the MM samples [Alvarez 2016] (b). AML + THYM represents the integration of acute myeloid leukemia and thymoma interactomes by metaVIPER [Ding 2018].



A training set comprising 42 samples from patients enrolled in STORM part 2 was assembled. Responders included Complete Response (sCR), Very Good Partial Response (VGPR), and Partial Response (PR) with DOCB > 36 days. Non-responders included Progressive Disease (PD) and Stable Disease (SD) samples treated longer than 30 days.

We inspected the homogeneity of the regulatory mechanisms associated with selinexor responder and non-responder phenotypic states. For this, regulatory protein activity signatures for each responder sample were obtained by comparison against the pool of selinexor non-responders (21 samples). Similarly, we obtained regulatory protein activity signatures for each non-responder sample by comparison against the pool of selinexor responders (21 samples). To evaluate the homogeneity of these signatures, we performed unsupervised hierarchical cluster analysis. This analysis indicated that responder and non-responder protein activity signatures are heterogeneous, and they potentially represent several distinct mechanisms of response, as well as three distinct mechanisms of resistance to selinexor treatment (Figure S7). Since at least 3 samples per mechanistic cluster are required for proper analysis, 5 samples from among the responders and 2 samples from the non-responders (highlighted by red circles in Figure S7), which were different from the rest of the samples in the same class, were removed from the training set. This left us 16 responders and 19 non-responders for further analysis.

Figure S7. Dendrograms showing the unsupervised clustering of the samples based on their similarity in MR signatures for patients that responded (a) and did not respond (b) to selinexor.



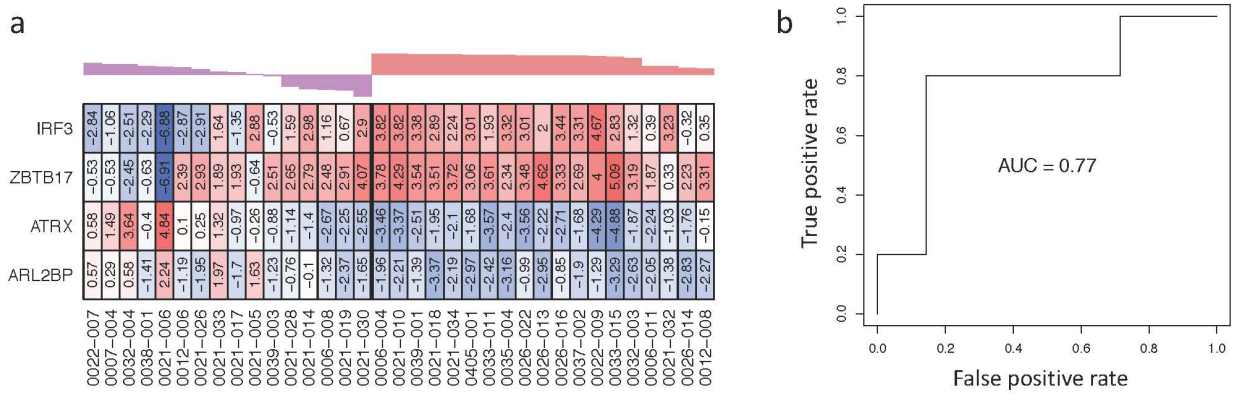
Based on the remaining 35 samples, we trained five classifiers—including Linear Discriminant Analysis (LDA) [Loh 1988], Logistic regression [Walker 1967], Neural Network [Bishop 2000], Random Forest [Brieman 2001], and Ridge regression methods [Hoerl 2000]. Using VIPER, a detailed inspection of the MR protein activity signatures characteristic of each responder and non-responder sample indicated

heterogeneity in the regulatory mechanisms leading to a selinexor resistant of susceptible tumor phenotype. The results from this analysis were useful for identifying distinct mechanisms leading to responder and non-responder phenotypic states and the samples representing them.

Results and Conclusions: The model performance was first tested using Leave-One-Out Cross Validation (LOOCV) analysis. LOOCV achieved best performance using the following top four Master Regulator proteins IRF3, ARL2BP, ZBTB17, and ATRX with Linear Discriminant Analysis (LDA) as the classifier. Area Under the receiver operating Curve (AUC) score is 0.862, with a confidence interval of [0.741, 0.982] , based on DeLong non-parametric method.

The performance of the trained model using classifier LDA was then tested on an independent, blinded set of samples, which were profiled as a separate batch, comprising 12 samples from MM patients enrolled in the STORM (part 1 and 2) trial. The analysis confirmed the values of the biomarkers as an effective classification metric, with AUC of 0.770, with a confidence interval of [0.456, 1.000], based on DeLong non-parametric method. Within the limitations imposed by a small testing cohort of only 12 samples, the selinexor Clinical Benefit (CB) biomarker correctly identified 4 out of 5 (80%) responder patients at a false positive rate of 20% (Supplementary Figure S8B) and misclassified only 1 out of 7 non-responder patients, yielding a prediction accuracy of 83%. Interestingly, the one misclassified non-responder showed a very high overall survival of 511 days. Due to the post hoc nature of this analysis, additional studies are required to validate the predictive capacity of this biomarker to predict clinical response to selinexor in patients with MM.

Figure S8. Selinexor clinical benefit biomarker for MM patients. (a) Heatmap showing the relative protein activity for the 4 prioritized MR proteins. The color bar above the heatmap shows the silhouette score for each sample, computed based on Euclidean distance, with Responder and Non-responder samples shown in purple and red, respectively. The values inside each cell in the heatmap shows the relative protein activity for each MR protein in each sample. (b) Evaluation of the biomarker performance in an independent sample set. Shown is the ROC analysis and estimated AUC.



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