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**Predicting failure of hematopoietic stem cell mobilization before it starts: the Predicted Poor Mobilizer (pPM) score**

**This is a pre print version of the following article:**

*Original Citation:*

*Availability:*

This version is available <http://hdl.handle.net/2318/1657273> since 2020-08-30T12:49:02Z

*Published version:*

DOI:10.1038/s41409-017-0051-y

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(Article begins on next page)

1 **TITLE:**

2 **Predicting failure of hematopoietic stem cell mobilization before it**  
3 **starts: the Predicted Poor Mobilizer (pPM) score.**

4 **RUNNING HEAD:** PREDICTING FAILURE OF HEMATOPOIETIC STEM CELL MOBILIZATION

5

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44 **MANUSCRIPT METRICS:** Abstract 200 words; Manuscript 4014 words. Tables 7; Figures 1; References 34  
45 Supplementary file 1.

46 **ABSTRACT**

47 Predicting mobilization failure before it starts may enable patient-tailored strategies. Although  
48 consensus criteria for predicted PM (pPM) are available, their predictive performance has never  
49 been measured on real data. We retrospectively collected and analyzed 1318 mobilization  
50 procedures performed for MM and lymphoma patients in the plerixafor era. In our sample,  
51 180/1318 (13.7%) were PM. The score resulting from published pPM criteria had sufficient  
52 performance for predicting PM, as measured by AUC (0.67, 95%CI: 0.63-0.72). We developed a  
53 new prediction model from multivariate analysis whose score (pPM-score) resulted in better AUC  
54 (0.80, 95%CI: 0.76-0.84,  $p < 0.0001$ ). pPM score included as risk factors: increasing age, diagnosis of  
55 NHL, positive bone marrow biopsy or cytopenias before mobilization, previous mobilization  
56 failure, priming strategy with G-CSF alone or without upfront plerixafor. A simplified version of  
57 pPM-score was categorized using a cut-off to maximize positive likelihood ratio (15.7, 95%CI: 9.9–  
58 24.8); specificity was 98% (95%CI: 97%-98.7%), sensitivity 31.7% (95%CI: 24.9%-39%); positive  
59 predictive value in our sample was 71.3% (95%CI: 60%-80.8%). Simplified pPM-score can “rule in”  
60 patients at very high risk for PM before starting mobilization, allowing changes in clinical  
61 management, such as choice of alternative priming strategies, to avoid highly likely mobilization  
62 failure.

63

64 **Introduction**

65 High dose chemotherapy followed by autologous stem cell rescue is a mainstay of treatment for  
66 Multiple Myeloma (MM), Non Hodgkin Lymphoma (NHL) and Hodgkin Disease (HD). Autologous  
67 stem cell transplant (auto-SCT) is almost exclusively performed today with peripheral blood stem  
68 cells (PBSCs) infusion; <sup>1</sup> therefore stem cell mobilization (SCM) currently represents a crucial step  
69 of the whole transplant process. A threshold of  $2 \times 10^6$  CD34+/Kg is regarded by most centers as the  
70 minimum amount of PBSCs to be infused to in order to safely perform the auto-SCT procedure.<sup>2</sup>  
71 Despite developments in SCM protocols, a proportion of patients between 5% and 30% fail to  
72 collect an adequate number of CD34+. <sup>3,4,5,6,7</sup> Poor mobilization forces the patient to undergo a re-  
73 mobilization procedure and in some cases leads to postponing or even abandoning a transplant  
74 strategy. Several factors have been associated with poor mobilization, <sup>4,5,8,9,10,11,18,12</sup> however a  
75 thorough profile of the patient at high risk of sub-optimal SCM is still missing.  
76 The Gruppo Italiano Trapianto Midollo Osseo (GITMO) has recently proposed a definition of the  
77 '*proven poor mobilizer* (PPM) and the *predicted poor mobilizer* (pPM), adopting a consensus based  
78 on an analytic hierarchy process (AHP).<sup>13</sup> While the GITMO definition of the PPM appears  
79 straightforward and is currently adopted by most European centers, identification of pPM appears  
80 more nuanced, and the GITMO pPM criteria should be validated in clinical trials and common  
81 clinical practice.  
82 Early identification of mobilization failure is even more important nowadays, given the availability  
83 of interventions to boost or rescue low-performing procedures, such as the CXCR4 antagonist  
84 plerixafor. Currently, low circulating CD34+ count before apheresis is widely accepted as the  
85 stronger parameter able to predict mobilization failure. Thus, to assist the clinician in a timely and  
86 cost-effective use of Plerixafor, various algorithms were developed, based on the circulating

87 CD34+ at day 4 (in case of steady-state mobilization) or at the time of white blood cell (WBC)  
88 recovery (in case of chemo-mobilization).<sup>14,15</sup> However, such algorithms are applicable belatedly,  
89 only a few hours before the apheresis procedure begins. Ideally, identification of patients at high  
90 risk of inadequate SCM should be performed before starting the mobilization process, and  
91 protocol planning should be individualized according to patient and disease characteristics, and to  
92 stem cell target dose. Such tailored approach might help to optimize resources management,  
93 avoiding suboptimal stem cell collection, need for re-mobilization, and redundant days of  
94 apheresis.

95 We therefore conducted this retrospective study with the aim to validate the predictive ability of  
96 GITMO criteria for pPM, by measuring their diagnostic accuracy for the outcome of mobilization  
97 failure. Furthermore, by analyzing SCM kinetics in a large cohort of myeloma and lymphoma  
98 patients, we aimed to improve their predictive ability by adding new data, in order to elaborate a  
99 “poor mobilization risk score” easily applicable in the everyday practice, to help decision-making  
100 and procedure customization based on pre-mobilization parameters.

## 101 **Methods**

102 This was a multicenter retrospective observational study involving 17 Italian GITMO centers. The  
103 protocol was approved by the Ethics Committee of Potenza and subsequently by all participating  
104 centers. A waiver of patient’s informed consent was obtained, owing to the retrospective nature  
105 of the study and provided that all patients’ data were collected and managed after being  
106 anonymized. The study was conducted in accordance to Helsinki declaration, Good Clinical  
107 Practice and of applicable national regulations. All Centers were asked to fill a database containing  
108 informations on all mobilization attempts performed between Jan 1<sup>st</sup> 2009 and Jan 31<sup>st</sup> 2014 in  
109 patients with Multiple Myeloma (MM), Hodgkin’s (HL) and non-Hodgkin’s Lymphomas (NHL).

110 Collected data pertained to patient's characteristics, underlying hematological disease,  
111 therapeutic history before mobilization and kinetics and results of the mobilization process; data  
112 collection was arranged in order to evaluate the presence or absence of GITMO criteria for pPM.

### 113 *Statistics*

114 The relevance of the candidate predictive factors was evaluated using univariate logistic  
115 regression for the outcome variable of pPM. Subsequently, multiple logistic regression with  
116 backward variable selection was performed to identify independent predictive factors. Explored  
117 variables are reported in table 1. WBC and absolute neutrophil counts were analyzed on the log-  
118 scale because of highly skewed distributions. Continuous parameters were not categorized a  
119 priori because this would have negatively affected the power of the analysis. Values of non-  
120 dichotomous categorical variables were transformed in dummy variables for the purpose of the  
121 analysis.

122 The outcome variable was the failure of a mobilization attempt defined according to the GITMO  
123 criteria for proven poor mobilizer. To this end, in patients treated with Plerixafor on demand, the  
124 data collected reflected the situation after the declaration of failure (i.e declining CD34+ cell count  
125 with a peak value  $<20/\text{mcl}$  or at least 3 aphereses with total collection  $<2 \times 10^6$  CD34/kg) and  
126 before Plerixafor administration. Conversely, patients treated with upfront Plerixafor had their  
127 data collected at the end of the mobilization process, as for all other patients.

128 To estimate the discriminating power of a chosen model, a receiver operating characteristic (ROC)  
129 curve was plotted. The areas under the ROC curves (AUCs) were calculated as previously  
130 described<sup>16</sup>. AUC comparisons were performed according to the method described by DeLong et  
131 al.<sup>17</sup>

132 Internal validation was performed applying the refined bootstrap described by Efron.<sup>18</sup> Random  
133 data splitting in training and validation sample was not performed because this internal validation  
134 procedure reduces the power for both model development and validation and is known to be  
135 inferior to bootstrap validation. Bootstrap validation used the AUC as performance index.

136 Two groups were defined by categorizing the score (linear predictor) of the final logistic  
137 regression model. For each cut-off, sensitivity, specificity, PPV, and NPV were calculated as simple  
138 proportions with 95% confidence intervals (CI). Likelihood ratios and their CI were calculated as  
139 ratios between proportions. The McNemar chi-square test was used to compare sensitivity and  
140 specificity between assays among failures and non-failures, respectively.<sup>19</sup> Cutpoint selection was  
141 based on clinical criteria: the purpose of the clinical tool for PPM prediction was to identify  
142 patients at very high risk for mobilization failure in order to support a practice-changing clinical  
143 decision. Therefore we aimed to maximize positive likelihood ratio (LR+) over negative likelihood  
144 ratio (LR-), by achieving a +LR value >10.

145 An explorative simplification of the final model was developed using basic mathematical  
146 operations. Spearman's rho was calculated to measure the correlation between the original score  
147 and the simplified version.<sup>20</sup>

148 Sample size calculation was based on AUC for the outcome variable of failed mobilization attempt  
149 (PPM): assuming a prevalence of PPM equal to 0.2, data from 845 mobilizations (169 failures) had  
150 to be collected to obtain an  $AUC \geq 0.57$  ( $\alpha=0.05$  and  $\text{power}=0.8$ ); with different PPM  
151 prevalences (0.1-0.5), the total number of mobilization attempts to be collected ranged from 530  
152 to 1600.

153 Statistical analyses were performed using Stata 12 (Statacorp, College Station, Texas) and  
154 MedCalc (MedCalc Software, Ostend, Belgium). Significance level was 0.05 for all analyses.

155

## 156 **Results**

### 157 *Patient characteristics*

158 We analyzed data from 1318 mobilization attempts. Disease distribution was the following: 600  
159 (46%) patients were affected by MM, 554 (42%) by NHL and 164 (12%) by HL. Median age at  
160 diagnosis was 56 years (range 5-76 years); four patients had less than 14 years at diagnosis but  
161 underwent mobilization after this age; 56% of patients were male. Sixty percent of patients had  
162 been treated with a single chemotherapy course before mobilization, 31% with 2 courses and 8%  
163 with 3 or more. Twelve percent of patients had been subjected to treatments potentially harmful  
164 to SCM (fludarabine, lenalidomide, radio-immunoconjugates, melphalan, carmustine); extensive  
165 radiotherapy on marrow bearing tissue had been used in 23 patients (1.7%). Before the  
166 mobilization attempt, 81% of patients were in partial or complete response; BM biopsy (BMB) was  
167 negative in 62% and showed extensive infiltration ( $\geq 30\%$  of total cellularity) in 3% of patients. Pre-  
168 mobilization BMB was omitted in 199 patients, due to different centers' policies (3 centers did not  
169 perform it routinely before mobilization).

170 Priming strategies involved the use of chemotherapy plus G-CSF in 94% of patients; chemotherapy  
171 protocols were quite disease-specific: cyclophosphamide was employed mostly in MM patients,  
172 while Ara-C containing regimens were preferred in NHL. Upfront plerixafor was added to the  
173 mobilization regimen in 44 patients (3%). Ninety-eight patients (7.4%) started SCM with at least  
174 one severe cytopenia ( $\geq$  grade 3 anemia, thrombocytopenia or neutropenia).



175 Overall, 180 patients (13.7%) failed the mobilization attempt, according to GITMO criteria for  
176 PPM. Failure resulted exclusively from inadequate CD34+ cells mobilization (peak CD34 count  
177 <20/mcl) in 36 cases (20%), from insufficient harvest (total CD34  $\leq 2 \times 10^6$ /kg) in 17 cases (9.4%)  
178 and from both criteria in 127 cases (70.6%). Further basal characteristics are reported in table 1.

179

#### 180 *Validation of the GITMO criteria*

181 To verify the actual consistency of GITMO consensus, we retrospectively applied the criteria to  
182 our cohort of 1318 cases. For each case, a score was generated (pPM-GITMO score) by summing 1  
183 point for each minor criteria and 2 points for each major criteria that were present. The only  
184 criterion considered in the original publication that could not be ascertained was BMB cellularity  
185 before mobilization, given the high rate of missing values.

186 This score ranged from 0 to 7 and the median value was 1. The AUC relative to the outcome of  
187 proven poor mobilizer was 0.673 (95%CI: 0.627-0.719, Fig. 1A). According to the GITMO  
188 consensus, the definition of pPM required at least one major criterion or two minor criteria;  
189 hence we considered a cut-off equal of greater than 2 for the pPM-GITMO score to be predictive.  
190 With this cut-off (Table 2), the sensitivity for the diagnosis of pPM was 53.3% (95%CI: 45.8%-  
191 60.8%) and the specificity 73.8% (95%CI: 71.2%-76.3%); LR+ was 2.04 (95%CI: 1.72-2.41). Given  
192 the prevalence of proven poor mobilizer observed in our cohort (13.7%), the PPV resulted 24.4%  
193 (95%CI: 20.2%-28.9%).

194 We implemented exploratory analyses to improve the predictive performance of the GITMO-pPM  
195 score. Increasing the cut-off to values equal or greater than 3 yielded a significantly lower  
196 sensitivity (39.4%) but higher specificity (90.8%); the PPV was 40.3% (95%CI: 33%-48%).

197 In the GITMO consensus, the splitting into major and minor criteria represented a simplification of  
198 the weights derived from AHP; thus we checked whether using the original AHP weights could  
199 improve the predictive performance of the GITMO-pPM score. Therefore we generated a score  
200 (AHP-pPM score) by summing the relative weight of each criterion as reported in the original  
201 publication. This score ranged from 0 to 0.55, had median value of 1 and produced an AUC of  
202 0.679 (95%CI: 0.634-0.725, Fig. 1B). To maximize specificity and LR+, we chose a cut-off equal of  
203 greater than 0.21 (Table 2), yielding a sensitivity of 33.3% (95%CI: 26.5%-40.7%) and a specificity  
204 of 93.1% (95%CI: 91.4%-94.5%); LR+ was 4.8 (95%CI: 3.57-6.46); PPV was 43.2%% (95%CI: 34.8%-  
205 51.8%).

#### 206 *Predictive factors for poor stem cell mobilization*

207 Sex, BMB at diagnosis and previous radiotherapy (local or extensive) did not show predictive  
208 relevance for mobilization failure in univariate analyses (Table 3). Among non-dichotomous  
209 categorical variables, BMB before mobilization had a significant protective effect if pathologic  
210 infiltration was absent, while it favored failure when disease infiltration reached 30% or more.  
211 NHL was strongly associated with failure, while HL was the opposite, and MM was non-significant;  
212 among priming strategies, use of G-CSF alone had strong impact on failure, while other  
213 chemotherapy regimens were not significant. Increasing age, number of full chemotherapy  
214 courses, previous use of fludarabine, lenalidomide, melphalan and carmustine, previous  
215 mobilization failure, refractory disease, and lower CBC values before mobilization, all had  
216 significant negative impact on the main outcome; upfront plerixafor use was instead associated  
217 with a reduced probability of failure.

#### 218 *Predicted Poor Mobilizer (pPM) score*

219 Hodgkin's lymphoma, refractory disease, absent pathologic infiltration at pre-mobilization BMB  
220 lost predictive relevance when evaluated in multivariate analysis. Continuous variables were  
221 categorized to help their potential application in clinical practice. For the same reason, the 4  
222 variables reporting for previous use of fludarabine, lenalidomide, melphan and carmustine were  
223 merged in one binary variable encoding for patients undergoing at least one of those treatment at  
224 risk. In the final model (Table 4), the following variables were identified as independent predictive  
225 factors for mobilization failure: increasing age (from ≤45 years to 46-60 years and to >60 years),  
226 diagnosis of NHL, disease infiltration ≥ 30% at the pre-mobilization BMB, previous mobilization  
227 failure, increasing number of full chemotherapy courses, previous treatment at risk (fludarabine,  
228 lenalidomide, melphan or carmustine), reduced hemoglobin (from >130 g/l to 80-130 g/l to less  
229 than 80 g/l), low WBC count (<5 x 10<sup>9</sup>/L), low Plt count (<170 x 10<sup>9</sup>/L), use of G-CSF alone as a  
230 priming strategy and not providing upfront Plerixafor. The predicted poor mobilizer score (pPM  
231 score) was calculated as shown in Table 5.

232 Predicted poor mobilizer score ranged from 2.46 to 12.82, had median value of 5.78 and  
233 produced an AUC of 0.801 (Fig. 1C; 95%CI: 0.764-0.838, Fig. 1C). We chose a cut-off >7.862 (Table  
234 2), yielding a specificity of 97.4% (95%CI: 96.3%-98.2%) and a sensitivity of 32.8% (95%CI: 26%-  
235 40.2%); LR+ was 12.43 (95%CI: 8.25-18.74), PPV was 66.3% (95%CI: 55.5%-76%).

236 The probability of mobilization failure according to the pPM score can be calculated as:

$$Probability = \frac{e^{(pPMscore-8.245)}}{e^{(pPMscore-8.245)} + 1}$$

237 The internal validation procedure correcting for overoptimism by bootstrap showed stability of  
238 predictive performance measured with AUC values (Table 6).

239 *Simplified predicted Poor Mobilizer score*

240 The classification according to the pPM-score involves some mathematical operations best  
241 performed using an electronic calculator. To make the score most practicable, we exploratively  
242 simplified it by rounding the weights of each factor to multiple of 0.5 points. This score was  
243 calculated as shown in Table 5.

244 The simplified version of the pPM score was highly correlated with the original one (Spearman's  
245 rho =0.983, p <0.0001). Simplified pPM score ranged from 2 to 10, had median value of 4.5 and  
246 produced an AUC of 0.795 (Fig. 1D; 95%CI: 0.757-0.833, Fig. 1C). We chose a cut-off  $\geq 6.5$  (Table  
247 2), yielding a specificity of 98% (95%CI: 97%-98.7%) and a sensitivity of 31.7% (95%CI: 24.9%-  
248 39%); LR+ was 15.7 (95%CI: 9.9-24.8); PPV was 71.3% (95%CI: 60%-80.8%).

249 *Score comparison*

250 The AUC of the 4 different scores were compared: GITMO-pPM and AHP-pPM score had both a  
251 significantly inferior AUC than pPM score and simplified pPM-score ( $p < 0.0001$  for all comparisons).  
252 There were no significant differences between GITMO-pPM and AHP-pPM score ( $p = 0.40$ ) and  
253 between pPM score and simplified pPM-score ( $p = 0.08$ ). Detailed results are reported in Tab S2.

254 We next compared the sensitivity and specificity of the different scores according to the chosen  
255 cut-offs: GITMO-pPM score with cut-off  $\geq 2$  had the best sensitivity compared to all alternatives;  
256 simplified pPM score with cut-off  $\geq 6.5$  had the best specificity with respect to all other models  
257 and cut-offs. Detailed results are reported in Tab S2.

258 **Discussion**

259 In this retrospective study, we collected a representative sample of mobilization outcomes in the  
260 plerixafor era in MM and lymphoma patients. The analysis of this large database aimed: 1-to  
261 validate published GITMO criteria for pPM (which were developed by AHP consensus method) on  
262 strong clinical data; 2-to improve the predictive ability of these criteria, by adding new variables  
263 and refining weights of already present criteria. The ultimate objective was to develop a  
264 standardized clinical tool able to identify “a priori” those patients at very high risk of failure,  
265 before starting the mobilization procedure, in order to drive a practice-changing clinical decision.  
266 Performance measures for prediction of mobilization failure were derived for 4 different models:  
267 (1) based on the original GITMO criteria (GITMO-pPM score); (2) using original AHP weights of  
268 GITMO criteria (AHP-pPM score); (3) a new model derived through multivariate regression analysis  
269 (pPM score); (4) a simplified version of this new model (simplified pPM score). The original GITMO  
270 criteria had modest performance measured by AUC (0.67); when applied with the proposed cut-  
271 off for pPM, it had limited sensitivity (53%) and modest specificity (74%) and use of original AHP  
272 weights did not improve their predictive performance. The new model (pPM-score) had far better  
273 AUC (0.80); its simplified version (ranging from 2 to 10) was categorized using a cut-off to  
274 maximize specificity: indeed in our sample, a high proportion of patients with simplified pPM-  
275 score >6.5 failed the mobilization (PPV =71%). Simplified pPM-score, combining unmodifiable  
276 patient-related factors with clinical choice-dependent variables, can be easily simulated before  
277 starting SCM, therefore supporting patient-tailored mobilization strategies..

278 Today, the first key decision in scheduling a first-line SCM regimen is the choice between a  
279 chemo-mobilization or a cytokine-only strategy. The second crucial stage is the dynamic  
280 identification of those patients, during SCM, in whom the addition of just-in-time plerixafor could  
281 be useful and cost-effective. To this end, different algorithms have been proposed.<sup>20,21,22</sup> all of

282 them include PB CD34+ cell count, the most reliable parameter to trigger plerixafor  
283 administration.<sup>23,24</sup> Other parameters proposed include WBC and platelet counts as surrogates of  
284 hematopoietic recovery, collection target dose and first day of apheresis yield.<sup>9</sup> Nevertheless, such  
285 algorithms present several limits. First of all, circulating CD34+ threshold values used to trigger  
286 plerixafor administration present a significant variability between different studies, ranging from  
287  $7^{27}$  to  $10^{9,15}$  or 20/mcl.<sup>25</sup> Secondly, most of those algorithms were not validated outside the  
288 institution they were developed, making problematic their application to other centers, as  
289 significant differences exist in facilities, staff, skills and procedures. In addition, many algorithms  
290 leave unresolved a “gray zone” with intermediate values of PB CD34+ (i.e. 10-20/mcl), where no  
291 recommendations are drawn and a “case by case” approach is suggested. The EBMT  
292 recommendations<sup>26</sup> recognized this window of uncertainty and proposed to fill the gap with a  
293 clinical decision taking into account risk factors for poor mobilization. Although these  
294 recommendations acknowledged first the role of patient and disease-related risk factors in the  
295 decision-making of mobilization, the choice was left to individual discretion. Recently published US  
296 recommendations<sup>27</sup> suggest as well to tailor the mobilization plan according to patient and disease  
297 characteristics; in case of MM, the authors suggest chemo-mobilization (instead of steady-state  
298 strategy) for patients previously treated with lenalidomide or melphalan, or having received more  
299 than 1 previous line of therapy. Similarly, for patient with lymphoma, the authors recommend to  
300 limit steady-state mobilization to patients “at low risk for mobilization failure”; once again, an  
301 explicit and reproducible clarification of the “risk of failure” is missing.

302 In 2012 a GITMO panel of experts proposed definitions for PM, recognizing two clearly different  
303 categories: the one of proven PM, which referred to a completed process merely requiring  
304 uniform and detailed characterization; the other one of predicted PM, i.e. a new classification of

305 patients expected to be at higher risk of failure for future mobilizations. Although application of  
306 AHP methodology to complex issues demonstrated excellent results even when consistent data  
307 were unavailable<sup>28</sup>, the adoption of a predictive model in a clinical setting requires nonetheless  
308 validation on real life patients. To verify the actual consistency of GITMO consensus, we  
309 retrospectively applied the criteria to our cohort of 1318 cases and measured their predictive  
310 performance with the AUC. Although no single measure of diagnostic accuracy fully captures the  
311 clinical value of a test, AUC is considered a valuable estimate of the global discriminative power,  
312 being independent from the chosen cut-off and from the disease prevalence.<sup>29</sup> Although the  
313 threshold to reach our predefined endpoint was set low (0.57), the obtained value (0.67) is  
314 considered indicative of sufficient diagnostic accuracy.

315 To gain a significant improvement over the GITMO criteria, we elaborated a new score based on  
316 data collected in our large database, evaluating all variables originally considered by the GITMO  
317 consensus and new ones: several risk factors previously identified by the Consensus were  
318 confirmed as relevant, such as stem cell poisons (e.g. lenalidomide and fludarabine). Instead, the  
319 role of extensive radiotherapy (previously considered major criterion) did not emerge as  
320 statistically significant, probably due to the very low number of patients who actually received it.  
321 Finally, a relevant statistical weight emerged for blood counts of all lineages, which adds to  
322 confirmation of other factors already identified by the Consensus (neoplastic BM involvement and  
323 previous chemotherapy burden), to allow detailed characterization of the BM functional reserve  
324 with simple parameters.

325 The pPM score undoubtedly improved the diagnostic accuracy with respect to GITMO criteria.  
326 However, improvements in test accuracy will not benefit patients unless they lead to changes in  
327 patient management.<sup>30</sup> To reach this goal, a clinical test should be easily applied and interpreted.

328 The pPM score contains predictors that are known before starting SCM: some pertain to patients'  
329 history, others to procedures routinely performed in the clinical practice (CBC, BMB), others to the  
330 mobilization planning. Algorithms based on PB CD34+ cell count only allow a late-stage clinical  
331 decision, when the mobilization process is already close to the end and only limited action  
332 (addition of just-in-time plerixafor) is possible. A finer and earlier planning would add a  
333 significantly wider range of possibilities to improve mobilization outcomes (table 7). To enhance  
334 feasibility of pPM-score, we created a simplified version, easily computable without an electronic  
335 calculator. Furthermore, we decided to dichotomize simplified pPM-score, aiming to make it a  
336 “ruling-in” diagnostic test. To this end we chose to maximize the LR+, albeit preserving a sensitivity  
337  $\geq 30\%$ . Many useful properties make LR+ suitable to this scope<sup>31</sup>: LR+ is independent of the disease  
338 prevalence in the examined group, making it immediately applicable to other clinical settings; LR+  
339 is considered the best indicator for ruling-in diagnosis: the higher the LR+, the greater is the shift  
340 of the probability of disease. Good diagnostic tests have LR+  $> 10$  and their positive result has a  
341 significant contribution to the diagnosis. The simplified pPM-score with a cut-off  $\geq 6.5$  had a LR+ of  
342 15.7: in our sample, this means that positive patients have their probability of failure increased  
343 from 13.7% to 71.3%, therefore requiring alternative strategies to avoid highly likely failure. One  
344 option is tailoring the priming strategy: the eternal dispute between steady-state and chemo-  
345 mobilization would be resolved if we could appropriately personalize mobilization strategies. Our  
346 results clearly support the use of chemo-mobilization in pPM, confirming a growing body of  
347 evidence.<sup>32</sup> Second, we provide a strong suggestion for upfront use of Plerixafor in pPM.  
348 Interestingly, very recently Yuan and colleagues<sup>33</sup> reported the results of the mobilization policy  
349 implemented in their center in Duarte, California. They propose the administration of upfront  
350 plerixafor in patients defined as “predicted poor mobilizers” according to criteria similar to ours,



351 and in MM patients candidates for tandem auto-SCT. Alternatively, the pPM-score can be  
352 integrated in algorithms based on PB CD34+ cell count, to resolve their “gray zones”, thus  
353 justifying earlier addition of Plerixafor. Finally, recognizing in advance pPMs will enable a special  
354 surveillance on them during the mobilization process, allowing for several technical optimizations  
355 such as use of large volume apheresis or starting apheresis with lower CD34+ thresholds (table 7).

356 Maximization of the LR+ implied an obvious reduction of the sensitivity (32% using cut-off  $\geq 6.5$ ):  
357 this important limitation should be taken into account if simplified pPM-score is used for clinical  
358 decisions. Negative patients should still be considered at risk for mobilization failure. In this group,  
359 we suggest careful monitoring of mobilization kinetics and application of algorithms for “just-in-  
360 time” Plerixafor to rescue additional patients from mobilization failure.

361 Another important issue to be considered when applying the pPM-score is that minimum dose of  
362 CD34+ is not equivalent to target dose: in our analysis, failure was defined as collection of less  
363 than  $2 \times 10^6$  CD34+/kg. However, in the clinical practice, definition of failure should be related to  
364 patient’s goals: as an example, collection of  $3 \times 10^6$  CD34+/kg is clearly unsatisfactory if a double  
365 auto-SCT is planned.

366 In conclusion, we have built on real large representative data a score to “rule in” patients at very  
367 high risk for PM before starting mobilization, allowing changes in clinical management, to avoid  
368 highly likely mobilization failure. To achieve the highest possible power from the available data,  
369 we performed internal validation by bootstrap,<sup>34</sup> thus confirming a high stability of the developed  
370 predictive model. Nevertheless, an external validation on an independent data set is still required  
371 to allow a broad application of this clinical tool. Finally, given the retrospective and observational  
372 nature of this study, it should be reminded that changes of SCM strategies which may be

373 suggested by pPM-score application (table 7), although reasonable, warrant to be tested in a  
374 prospective interventional trial to demonstrate clinical effectiveness.

375 **Conflicts of interest:**

376 The authors do not have any conflicts of interest.

377 **Acknowledgments:**

378 The authors are very grateful to Giuseppe Ausoni, Paola Brambilla, Saveria Capria, Gloria  
379 Margiotta Casaluci, Michele Cimminiello, Annarita Conconi, Carmela Cuomo, Katia Codeluppi,  
380 Mario Delia, Roberta Distefano, Annalisa Di Marco, Luca Facchini, Salvatore Gattillo, Maria Gozzer,  
381 Svitlana Gumenyuk, Francesco Marchesi, Giovanna Meloni, Angela Melpignano, Luca Nassi,  
382 Domenico Pastore, Giuseppe Pietrantuono, Michele Pizzuti, Giovanni Quarta, Azzurra Anna  
383 Romeo, Federica Sorà, Andrea Spadaro and Stefania Trinca for their contribution to this study.

384 **Author contributions**

385 JO performed the statistical analysis and wrote the manuscript; AO contributed to study design,  
386 interpreted the results, contributed to manuscript writing, reviewed and approved the  
387 manuscript; EDN contributed to study design and to the statistical analysis; FS contributed to data  
388 collection and interpretation of the results, approved and edited the manuscript; IA, MC, MP, PP,  
389 PEP contributed to data collection and interpretation of the results; PC, LF, GG, LN, SS, NP, MM,  
390 TM, MP, FZ, FC, SM, AM, PM, SC, FM, KC, GM, FL, GS, DP, GM contributed to contributed to  
391 patient care and data collection.

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## Figure Legends

Figure 1: Area Under the Receiving Operating Characteristic (ROC) Curve (AUC) for the outcome of proven poor mobilizer and the 4 scores generated (A: GITMO-PPM score; B: AHP-PPM score; C: PPM score; D: simplified PPM score).

## REFERENCES

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- <sup>1</sup>Passweg JR, Baldomero H, Bader P, Bonini C, Cesaro S, Dreger P, et al. Hematopoietic stem cell transplantation in Europe 2014: more than 40 000 transplants annually. *Bone Marrow Transplant* 2016; 51: 786-92.
- <sup>2</sup>Weaver CH, Hazelton B, Birch R, Palmer P, Allen C, Schwartzberg L et al. An analysis of engraftment kinetics as a function of the CD34 content of peripheral blood progenitor cell collections in 692 patients after the administration of myeloablative chemotherapy. *Blood* 1995; 86: 3961–3969.
- <sup>3</sup>Hubel K, Fresen MM, Apperley JF, Basak GW, Douglas KW, Gabriel IH et al. European data on stem cell mobilization with plerixafor in non-Hodgkin's lymphoma, Hodgkin's lymphoma and multiple myeloma patients. A subgroup analysis of the European Consortium of stem cell mobilization. *Bone Marrow Transplant* 2012; 47: 1046-50.
- <sup>4</sup>Perseghin P, Terruzzi E, Dassi M, Baldini V, Parma M, Coluccia P et al. Management of poor peripheral blood stem cell mobilization: incidence, predictive factors, alternative strategies and outcome. A retrospective analysis on 2177 patients from three major Italian institutions. *Transfus Apher Sci* 2009; 41: 33–37.
- <sup>5</sup>Pusic I, Jiang SY, Landua S, Uy GL, Rettig MP, Cashen AF, et al. Impact of mobilization and remobilization strategies on achieving sufficient stem cell yields for autologous transplantation. *Biol Blood Marrow Transplant* 2008; 14: 1045–1056.
- <sup>6</sup>Milone G, Martino M, Spadaro A, Leotta S, Di Marco A, Scalzulli P et al. Plerixafor on demand combined with chemotherapy and granulocyte colony-stimulating factor: significant improvement in

---

peripheral blood stem cells mobilization and harvest without increase in costs. *Br J Haematol* 2013; 164: 113-23

<sup>7</sup>Farina L, Guidetti A, Spina F, Roncari L, Longoni P, Ravagnani F et al. Plerixafor 'on demand': results of a strategy based on peripheral blood CD34<sup>+</sup> cells in lymphoma patients at first or subsequent mobilization with chemotherapy + G-CSF. *Bone Marrow Transplant* 2014; 9: 453-5.

<sup>8</sup>Sancho JM, Morgades M, Grifols JR, Juncà J, Guardia R, Vives S et al. Predictive factors for poor peripheral blood stem cell mobilization and peak CD34<sup>+</sup> cell count to guide pre-emptive or immediate rescue mobilization. *Cytotherapy* 2012; 4: 823–829.

<sup>9</sup>Lanza F, Lemoli RM, Olivieri A, Laszlo D, Martino M, Specchia G et al. Factors affecting successful mobilization with plerixafor: an Italian prospective survey in 15 patients with multiple myeloma and lymphoma. *Transfusion* 2013; 54: 31–339.

<sup>10</sup>Sinha S, Gertz MA, Lacy MQ, Dispenzieri A, Hayman SR, Buadi FK et al. Majority of patients receiving initial therapy with lenalidomide-based regimens can be successfully mobilized with appropriate mobilization strategies. *Leukemia* 2012; 26: 1119–1122.

<sup>11</sup>Giralt S, Stadtmauer EA, Harousseau JL, Palumbo A, Bensinger W, Comenzo RL, et al. International Myeloma Working Group (IMWG) consensus statement and guidelines regarding the current status of stem cell collection and high-dose therapy for multiple myeloma and the role of plerixafor (AMD 3100). *Leukemia* 2009; 23: 1904–12.

- 
- <sup>12</sup>Attolico I, Pavone V, Ostuni A, Rossini B, Musso M, Crescimanno A et al. Plerixafor added to chemotherapy plus G-CSF is safe and allows adequate PBSC collection in predicted poor mobilizer patients with multiple myeloma or lymphoma. *Biol Blood Marrow Transplant* 2012; 18: 241– 249.
- <sup>13</sup>Olivieri A, Marchetti M, Lemoli R, Tarella C, Iacone A, Lanza F et al. Proposed definition of 'poor mobilizer' in lymphoma and multiple myeloma: an analytic hierarchy process by ad hoc working group Gruppo Italiano Trapianto di Midollo Osseo. *Bone Marrow Transplant* 2012; 47: 342–351.
- <sup>14</sup>Abhyankar S, DeJarnette S, Aljitawi O, Ganguly S, Merkel D, McGuirk J. Risk-based approach to optimize autologous hematopoietic stem cell (HSC) collection with the use of plerixafor. *Bone Marrow Transplant* 2012; 47:83–487.
- <sup>15</sup>Costa LJ, Alexander ET, Hogan KR, Schaub C, Fouts TV, Stuart RK. Development and validation of a decision-making algorithm to guide the use of plerixafor for autologous hematopoietic stem cell mobilization. *Bone Marrow Transplant* 2011; 6: 64–69.
- <sup>16</sup> Hanley JA, McNeil BJ. The meaning and use of the area under a receiver operating characteristic (ROC) curve. *Radiology* 1982; 143: 29-36.
- <sup>17</sup> DeLong, E. R., D. M. DeLong, and D. L. Clarke-Pearson. 1988. Comparing the areas under two or more correlated receiver operating characteristic curves: A nonparametric approach. *Biometrics* 44: 837–845.
- <sup>18</sup>Efron B, Tibshirani RJ. *An Introduction to the Bootstrap*. Vol. 57: Chapman & Hall/CRC; 1994.
- <sup>19</sup>Trajman A, Luiz RR. McNemar chi2 test revisited: comparing sensitivity and specificity of diagnostic examinations. *Scand J Clin Lab Invest*. 2008;68(1):77-80.

- 
- <sup>20</sup>Hooster E, Dreyling M, Klapper W, Gisselbrecht C, van Hoof A, Kluin-Nelemans HC, et al. A new prognostic index (MIPI) for patients with advanced-stage mantle cell lymphoma. *Blood* 2008; 111: 558-65.
- <sup>21</sup>Micallef IN, Sinha S, Gastineau DA, Wolf R, Inwards DJ, Gertz MA et al. Cost-effectiveness analysis of a risk-adapted algorithm for plerixafor use in autologous peripheral blood stem cell mobilization. *Biol Blood Marrow Transplant*. 2013;19: 87-93.
- <sup>22</sup>Horwitz ME, Chute JP, Gasparetto C, Long GD, McDonald C, Morris A et al. Preemptive dosing of plerixafor given to poor stem cell mobilizers on day 5 of G-CSF administration. *Bone Marrow Transplant* 2012; 47: 1051-5.
- <sup>23</sup>Gambell P, Herbert K, Dickinson M, Stokes K, Bressel M, Wall D et al. Peripheral blood CD34+cell enumeration as a predictor of apheresis yield: an analysis of over 1000 collections. *Biol Blood Marrow Transplant*. 2012;18:763-772.
- <sup>24</sup>Pierelli L, Perseghin P, Marchetti M, Accorsi P, Fanin R, Messina C, et al. Best practice for peripheral blood progenitor cell mobilization and collection in adults and children: results of a Società Italiana Di Emaferesi e Manipolazione Cellulare (SIDEM) and Gruppo Italiano Trapianto Midollo Osseo (GITMO) consensus process. *Transfusion* 2012; 52: 893-905.
- <sup>25</sup>Chow E, Rao KV, Wood WA, Covington D, Armistead PM, Coghill J, et al. Effectiveness of an algorithm-based approach to the utilization of plerixafor in patients undergoing chemotherapy-based stem cell mobilization. *Biol Blood Marrow Transplant*. 2014; 20: 1064-8.

- 
- <sup>26</sup>Mohty M, Hübel K, Kröger N, Aljurf M, Apperley J, Basak GW, et al. Autologous haematopoietic stem cell mobilisation in multiple myeloma and lymphoma patients: a position statement from the European Group for Blood and Marrow Transplantation. *Bone Marrow Transplant* 2014; 49: 865-72.
- <sup>27</sup>Giralt S, Costa L, Schriber J, Dipersio J, Maziarz R, McCarty J, et al. Optimizing autologous stem cell mobilization strategies to improve patient outcomes: consensus guidelines and recommendations. *Biol Blood Marrow Transplant* 2014; 20: 295-308.
- <sup>28</sup>Whitaker R. Validation examples of the analytic hierarchy process and analytic network process. *Mathematical and Computer Modelling* 2007; 46: 840-859.
- <sup>29</sup>Simundić AM. Measures of Diagnostic Accuracy: Basic Definitions. *EJIFCC*. 2009;19(4):203-11.
- <sup>30</sup>Ferrante di Ruffano L, Hyde C, McCaffery KJ, Bossuyt PM, Deeks JJ. Assessing the value of diagnostic tests: a framework for designing and evaluating trials. *BMJ* 2012; 344: e686
- <sup>31</sup>Grimes DA, Schulz KF. Refining clinical diagnosis with likelihood ratios. *Lancet* 2005; 365: 1500-5.
- <sup>32</sup>Olivieri A, Saraceni F. Mobilization policy in multiple myeloma: minimum target or law of redundancy? Two different approaches by the two sides of the Atlantic Ocean. *Bone Marrow Transplant* 2016; 51: 348-50.
- <sup>33</sup>Yuan S, Wang S. How do we mobilize and collect autologous peripheral blood stem cells? *Transfusion* 2017; 57: 13-23.
- <sup>34</sup>Steyerberg EW, Harrell FE, Jr., Borsboom GJ, Eijkemans MJ, Vergouwe Y, Habbema JD. Internal validation of predictive models: efficiency of some procedures for logistic regression analysis. *J Clin Epidemiol* 2001; 54: 774-781



Figure 1: Area Under the Receiving Operating Characteristic (ROC) Curve (AUC) for the outcome of proven poor mobilizer and the 4 scores generated (A: GITMO-PPM score; B: AHP-PPM score; C: PPM score; D: simplified PPM score).

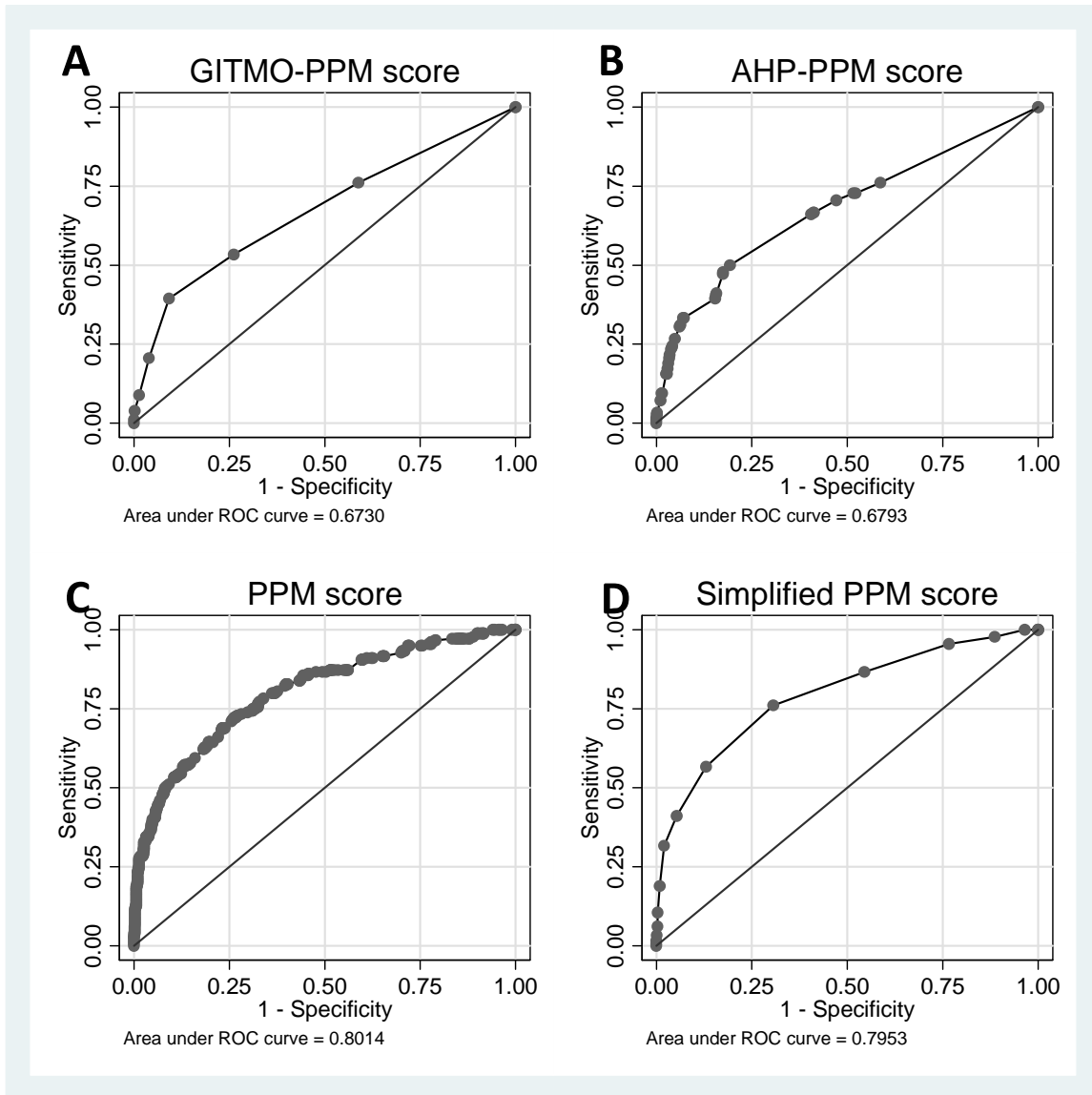


Table I: Basal characteristics

<b>BASAL CHARACTERISTICS</b>	<b>ALL PATIENTS</b>	<b>MM</b>	<b>NHL</b>	<b>HL</b>
<b>Age at diagnosis, median (range)</b>	55.6 (4.6 - 76.5)	59 (18 - 74)	54 (5 - 76)	37 (5 - 76)
≤45	337 (26%)	57 (10%)	166 (30%)	114 (70%)
45-60	571 (43%)	293 (49%)	246 (44%)	32 (20%)
> 60	410 (31%)	250 (42%)	142 (26%)	18 (11%)
<b>Sex</b>				
Male	753 (57%)	321 (54%)	346 (62%)	86 (52%)
Female	565 (43%)	279 (47%)	208 (38%)	78 (48%)
<b>Disease</b>				
Multiple Myeloma	600 (46%)			
Non-Hodgkin's Lymphoma	554 (42%)			
Hodgkin's Lymphoma	164 (12%)			
<b>Bone marrow infiltration at diagnosis</b>				
Absent	499 (38%)	18 (3%)	332 (60%)	149 (91%)
Present: < 30%	306 (23%)	174 (29%)	121 (22%)	11 (7%)
Present: ≥ 30%	511 (39%)	407 (68%)	100 (18%)	4 (2%)
Unknown	2 (0.2%)	1 (0.2%)	1 (0.2%)	0 (0%)
<b>PREVIOUS TREATMENTS</b>				
<b>Number of chemotherapy courses - median (range)</b>	1 (1 - 6)	1 (1 - 6)	2 (1 - 6)	2 (1 - 5)
1	790 (60%)	489 (82%)	259 (47%)	42 (26%)
2	413 (31%)	79 (13%)	230 (42%)	104 (63%)
3	93 (7%)	21 (4%)	57 (10%)	15 (9%)
≥4	22 (2%)	11 (2%)	8 (1%)	3 (2%)
<b>Use of myelotoxic agents (at least one)</b>	153 (12%)	133 (22%)	15 (3%)	5 (3%)
Fludarabine	12 (1%)	1 (0.2%)	8 (1%)	3 (2%)
Lenalidomide (≤ 4cycles / > 4 cycles)	114 (9%) / 7 (1%)	113 (19%) / 7 (1%)	1 (0.2%) / 0 (0%)	0 (0%) / 0 (0%)
Radioimmunoconjugates	1 (0.1%)	1 (0.2%)	0 (0%)	0 (0%)
Melphalan	27 (2%)	20 (3%)	5 (1%)	2 (1%)
BCNU	9 (1%)	2 (0.3%)	5 (1%)	2 (1%)
Radiotherapy (limited / extensive)	122 (9%) / 23 (2%)	42 (7%) / 21 (4%)	37 (7%) / 6 (1%)	43 (26%) / 5 (3%)
<b>Bone marrow infiltration before mobilization</b>				
Absent	821 (62%)	201 (34%)	471 (85%)	149 (91%)

<b>Present: &lt; 30%</b>	263 (20%)	231 (39%)	26 (5%)	6 (4%)
<b>Present: ≥ 30%</b>	35 (3%)	30 (5%)	5 (1%)	0 (0%)
<b>Unknown</b>	199 (15%)	138 (23%)	52 (9%)	9 (5%)
<b>Disease status at mobilization</b>				
<b>Remission (complete or partial)</b>	1066 (81%)	535 (89%)	426 (77%)	105 (64%)
<b>Refractory</b>	242 (18%)	63 (11%)	121 (22%)	58 (35%)
<b>Unknown</b>	10 (1%)	2 (0.3%)	7 (1%)	1 (1%)
<b>Failed previous mobilization attempt</b>	94 (7%)	37 (6%)	51 (9%)	6 (4%)
<b>MOBILIZATION</b>				
<b>Blood count values before starting mobilization</b>				
<b>Hemoglobin (g/dl) - median (range)</b>	11.8 (7.2 - 19.8)	12.2 (7.2 - 18.8)	11.3 (7.2 - 17.3)	11.6 (7.9 - 16.2)
<b>Leukocytes (x 10<sup>9</sup>/L) - median (range)</b>	5.2 (0 - 426)	5.3 (0.3 - 42.6)	4.9 (0 - 58.27)	5.8 (0.9 - 22.8)
<b>Neutrophils (x 10<sup>9</sup>/L) - median (range)</b>	3.2 (0 - 282)	3.1 (0.1 - 28.2)	3.1 (0 - 45.2)	3.9 (0.1 - 18.7)
<b>Platelets (x 10<sup>9</sup>/L) - median (range)</b>	223 (6 - 1167)	230 (6 - 665)	202 (6 - 1167)	239 (7 - 601)
<b>Mobilization regimen</b>				
<b>High dose CTX (2-7 g/mq) + G-CSF</b>	650 (49%)	499 (83%)	131 (24%)	20 (12%)
<b>DHAP + G-CSF</b>	126 (10%)	6 (1%)	101 (18%)	19 (12%)
<b>IEV + G-CSF</b>	70 (5%)	0 (0%)	21 (4%)	49 (30%)
<b>High dose Ara-C + G-CSF</b>	107 (8%)	4 (1%)	100 (18%)	3 (2%)
<b>Other chemotherapy regimen + G-CSF</b>	292 (22%)	43 (7%)	177 (32%)	72 (44%)
<b>G-CSF alone</b>	73 (6%)	48 (8%)	24 (4%)	1 (1%)
<b>Dose of G-CSF (µg/kg)</b>				
<b>5</b>	904 (69%)	332 (55%)	441 (80%)	131 (80%)
<b>10</b>	413 (31%)	267 (45%)	113 (20%)	33 (20%)
<b>15</b>	1 (0.1%)	1 (0.2%)	0 (0%)	0 (0%)
<b>Plerixafor administered (upfront)</b>	44 (3%)	18 (3%)	24 (4%)	2 (1%)
<b>Peak CD34+ value (cells/mcl) - median (range)</b>				
<b>&lt;5</b>	61 (5%)	20 (3%)	36 (6%)	5 (3%)
<b>&lt;20</b>	163 (12%)	66 (11%)	89 (16%)	8 (5%)
<b>Number of aphereses - median (range)</b>				
<b>&gt;3</b>	47 (4%)	32 (5%)	10 (2%)	5 (3%)
<b>Total harvest (x 10<sup>6</sup> CD34+/kg) - median (range)</b>				
<b>&lt;1</b>	118 (9%)	47 (8%)	64 (12%)	7 (4%)

<2	144 (11%)	59 (10%)	76 (14%)	9 (5%)
2 – 5	204 (15%)	87 (15%)	93 (17%)	24 (15%)
>5	970 (74%)	454 (76%)	385 (69%)	131 (80%)
<b>Failed mobilization</b>	180 (14%)	75 (13%)	95 (17%)	10 (6%)
<b>Due to low CD34+ peak count</b>	163 (12%)	66 (11%)	89 (16%)	8 (5%)
<b>Due to insufficient harvest</b>	144 (11%)	56 (9%)	76 (14%)	9 (5%)
<b>Due to both above criteria</b>	127 (10%)	50 (8%)	70 (13%)	7 (4%)

Table II: Measures of sensitivity, specificity, positive (LR+) and negative (LR-) likelihood ratio, diagnostic odds ratio, positive (PPV) and negative (NPV) predictive values for selected cut-offs of the 4 scores generated to predict mobilization failure.

Cut-off	GITMO-PPM score		AHP-PPM score	PPM score		Simplified PPM score	
	≥ 2	≥ 3	≥ 0.21	> 7.48	> 7.862	≥ 6	≥ 6.5
<b>Sensitivity (SE)</b>	0.533 (0.458 - 0.608)	0.394 (0.323 - 0.47)	0.333 (0.265 - 0.407)	0.4 (0.328 - 0.476)	0.328 (0.26 - 0.402)	0.411 (0.338 - 0.487)	0.317 (0.249 - 0.39)
<b>Specificity (SP)</b>	0.738 (0.712 - 0.763)	0.908 (0.889 - 0.924)	0.931 (0.914 - 0.945)	0.952 (0.938 - 0.963)	0.974 (0.963 - 0.982)	0.947 (0.933 - 0.96)	0.98 (0.97 - 0.987)
<b>Positive Likelihood Ratio (LR+)</b>	2.04 (1.72 - 2.41)	4.28 (3.31 - 5.53)	4.8 (3.57 - 6.46)	8.28 (6.05 - 11.33)	12.43 (8.25 - 18.74)	7.8 (5.76 - 10.55)	15.67 (9.91 - 24.77)
<b>Negative Likelihood Ratio (LR-)</b>	0.63 (0.54 - 0.74)	0.67 (0.59 - 0.75)	0.72 (0.65 - 0.8)	0.63 (0.56 - 0.71)	0.69 (0.62 - 0.76)	0.62 (0.55 - 0.7)	0.7 (0.63 - 0.77)
<b>Diagnostic Odds Ratio</b>	3.22 (2.34 - 4.44)	6.41 (4.47 - 9.18)	6.7 (4.57 - 9.84)	13.13 (8.79 - 19.62)	18.01 (11.2 - 28.96)	12.54 (8.46 - 18.59)	22.47 (13.42 - 37.58)
<b>Positive predictive value (PPV)</b>	0.244 (0.202 - 0.289)	0.403 (0.33 - 0.48)	0.432 (0.348 - 0.518)	0.567 (0.476 - 0.655)	0.663 (0.555 - 0.76)	0.552 (0.464 - 0.638)	0.713 (0.6 - 0.808)
<b>Negative predictive value (NPV)</b>	0.909 (0.889 - 0.927)	0.905 (0.886 - 0.921)	0.898 (0.88 - 0.915)	0.909 (0.892 - 0.925)	0.902 (0.884 - 0.918)	0.91 (0.893 - 0.926)	0.901 (0.883 - 0.917)



Table III: Association to mobilization failure according to univariate logistic regression

Candidate predictive factor	Odds ratio (95% CI)	Probability (Wald test)
Age (years)	1.01 (1 - 1.03)	0.033
Sex (female)	1.23 (0.89 - 1.68)	0.204
<b>Disease</b>		
MM	0.83 (0.61 - 1.15)	0.264
NHL	1.65 (1.21 - 2.27)	0.002
HL	0.38 (0.19 - 0.73)	0.004
<b>BMB at diagnosis</b>		
Absent	0.99 (0.72 - 1.37)	0.967
<30%	0.71 (0.48 - 1.06)	0.094
≥ 30%	1.27 (0.93 - 1.75)	0.135
<b>Number of chemotherapy courses</b>	2.03 (1.69 - 2.45)	<0.001
Previous use of BCNU	5.15 (1.37 - 19.35)	0.015
Previous use of Fludarabine	4.61 (1.45 - 14.69)	0.010
Previous use of Melphalan	7.29 (3.37 - 15.79)	<0.001
<b>Previous use of Lenalidomide</b>		
Absent	0.43 (0.28 - 0.68)	<0.001
≤ 4 cycles	2.25 (1.42 - 3.57)	0.001
>4 cycles	2.55 (0.49 - 13.22)	0.266
<b>At least one treatment at risk</b>	2.93 (1.98 - 4.35)	<0.001
<b>Previous radiotherapy</b>		
Absent	0.89 (0.55 - 1.43)	0.623
Limited	1.1 (0.65 - 1.87)	0.711
Extensive (on marrow bearing tissue)	1.18 (0.45 - 3.09)	0.743
<b>Previous mobilization failure</b>	6.36 (4.08 - 9.9)	<0.001
<b>Disease remission (CR or PR) before mobilization</b>	1.96 (1.37 - 2.81)	<0.001
<b>Pre-mobilization BMB</b>		
Absent	0.74 (0.54 - 1.02)	0.066
<30%	1.04 (0.71 - 1.54)	0.828
≥ 30%	3.02 (1.45 - 6.28)	0.003
Notdone	1.2 (0.79 - 1.83)	0.392
<b>CBC before mobilization</b>		
Hemoglobin (10 g/L)	0.81 (0.74 - 0.9)	<0.001
Leukocytes (10-fold)	0.31 (0.18 - 0.55)	<0.001
Neutrophils (10-fold)	0.39 (0.24 - 0.64)	<0.001
Platelets (1 x 10 <sup>9</sup> /L)	0.996 (0.994 - 0.997)	<0.001
<b>Priming strategy</b>		
CTX 3-7 g/m <sup>2</sup> + G-CSF	0.78 (0.57 - 1.07)	0.118
DHAP or DHAox + G-CSF	0.58 (0.31 - 1.1)	0.094
IEV + G-CSF	0.58 (0.25 - 1.36)	0.208
High-dose Ara-C + G-CSF	0.95 (0.53 - 1.7)	0.857
Other chemotherapy + G-CSF	0.97 (0.66 - 1.42)	0.865
G-CSF alone	5.43 (3.31 - 8.9)	<0.001

Type of G-CSF		
<b>Lenograstim</b>	0.9 (0.65 - 1.25)	0.536
<b>Filgrastim</b>	1.23 (0.9 - 1.69)	0.197
<b>Pegfilgrastim</b>	1.47 (0.41 - 5.2)	0.553
<b>Biosimilar</b>	0.79 (0.18 - 3.46)	0.752
<b>Missing data</b>	0.63 (0.32 - 1.23)	0.179
<b>Double G-CSF dose (vs standard)</b>	1.02 (0.73 - 1.43)	0.918
<b>Upfront plerixafor</b>	2.47 (1.25 - 4.89)	0.010



Table IV: Independent predictive factors for mobilization failure identified by backward variable selection with multiple logistic regression on significance level 0.1 for the Wald statistic

Predictive factor	$\beta$	Odds ratio (95% CI)	Probability (Wald test)
Age class (46-60 years = 1; > 60 years = 2)	0.3796	1.46 (1.14 - 1.88)	0.003
Diagnosis = NHL	0.5535	1.74 (1.16 - 2.6)	0.007
Disease infiltration $\geq$ 30% at the pre-mobilization BMB	1.269	3.56 (1.51 - 8.35)	0.004
Number of full chemotherapy courses	0.5888	1.8 (1.43 - 2.27)	<0.001
At least one previous treatment at risk	0.7739	2.17 (1.28 - 3.67)	0.004
Pre-mobilization Hb value class (<80 g/l = 1; 80 – 130 g/l = 2)	1.1165	3.05 (1.72 - 5.42)	<0.001
Pre-mobilization WBC < 5 x 10 <sup>9</sup> /L	0.7185	2.05 (1.41 - 2.99)	<0.001
Pre mobilization Plt< 170 x 10 <sup>9</sup> /L	0.5869	1.8 (1.23 - 2.62)	0.002
Priming with G-CSF alone	2.2513	9.5 (4.75 - 19)	<0.001
Upfront Plerixafor not planned	2.7292	15.32 (5.09 - 46.16)	<0.001
Previous mobilization failure	1.9059	6.73 (3.67 - 12.34)	<0.001

Table V. Calculation of *predicted Poor Mobilizer (pPM)* and *simplified predicted Poor Mobilizer (s-PPM )score*

pPM score	Simplified pPM score
0.3796 (if age 46-60 years)	0.5 (if age > 60 years)
+ 0.7592 (if age > 60 years)	+ 0.5 (if diagnosis NHL)
+ 0.5535 (if diagnosis NHL)	+ 1 (if disease infiltration $\geq$ 30% at the pre-mobilization BMB)
+ 1.269 (if disease infiltration $\geq$ 30% at the pre-mobilization BMB)	+ 0.5 x [number of full chemotherapy courses]
+ 0.5888 x [number of full chemotherapy courses]	+ 0.5 (if one previous treatment at risk)
+ 0.77388929 (if one previous treatment at risk)	+ 1 (if Hb 80 – 130 g/l)
+ 1.1165 (if Hb 80 – 130 g/l)	+ 2 (if Hb < 80 g/l)
+ 2.233 (if Hb < 80 g/l)	+ 0.5 (if WBC count < 5 x 10 <sup>9</sup> /L)
+ 0.7185 (if WBC count < 5 x 10 <sup>9</sup> /L)	+ 0.5 (if Plt < 170 x 10 <sup>9</sup> /L)
+ 0.5869 (if Plt < 170 x 10 <sup>9</sup> /L)	+ 2 (if priming with G-CSF alone)
+ 2.251 (if priming with G-CSF alone)	+ 2 (if upfront Plerixafor not planned)
+ 2.7292 (if upfront Plerixafor not planned)	+ 1.5 (if previous mobilization failure)
+ 1.906 (if previous mobilization failure)	

Table VI: Bootstrap validation according to Efron et al.

Apparent area under the ROC curve	0.8014
Mean AUC of 10.000 bootstrap samples	0.8066
Mean AUC of 10.000 tests in original database	0.7959
Optimism in apparent performance	0.0107
Optimism-corrected AUC	0.7907

Table 7. The pPM score can be used to tailor SCM strategy from baseline, unmodifiable risk factors: here we describe examples of calculation of pPM score in 10 different clinical scenarios; we also report suggested SCM strategies based on changes of pPM score due to different clinical choices.

Category of patients	Baseline pPM score	Suggested SCM strategy	pPM score	Predicted probability of failure	Other suggestions
<b>Low risk</b>					
<b>MM with <math>\geq</math> PR after 1<sup>st</sup> line (without lenalidomide), no cytopenias, even beyond 60y</b>	1	Cytokine-only (+2) No upfront PLX (+2)	5	<15%	Tailor collection according to target dose
<b>HL without significant BM involvement after 2<sup>nd</sup> line, &lt;60y</b>	1		5	<15%	
<b>NHL without significant BM involvement after 1<sup>st</sup> line, &lt;60y</b>	1		5	<15%	
<b>Intermediate risk</b>					
<b>NHL without significant BM involvement after 1<sup>st</sup> salvage treatment, mild cytopenias*, &gt; 60y</b>	3	CHT-based SCM (0) No upfront PLX (+2)	5	12-16%	Careful SCM monitoring with prompt intervention (Plx on demand)
<b>MM with marrow plasmacytosis &lt;30% after 2<sup>nd</sup> line with lenalidomide, mild cytopenias*, &gt; 60y</b>	3		5	15-18%	
<b>MM with marrow plasmacytosis &lt;30% after 2<sup>nd</sup> line with lenalidomide, no cytopenias, after failed SCM attempt, &gt; 60y</b>	3.5	Cytokine-only (+2) Upfront PLX (0)	5.5	<30%	
<b>High risk</b>					
<b>NHL (no significant BM involvement) after 1<sup>st</sup> salvage treatment, after failed SCM attempt, mild cytopenias*, &gt; 60y</b>	4.5	CHT-based SCM (0) No upfront PLX (+2)	6.5	50%	On demand Plx (if not planned upfront)
		CHT-based SCM (0) Upfront PLX (0)	4.5	<10%**	
<b>MM with marrow plasmacytosis &gt;30% after 2<sup>nd</sup> line with lenalidomide, trilinear cytopenias, &gt; 60y</b>	5	CHT-based SCM (0) No upfront PLX (+2)	7	71%	Use of large volume apheresis
		CHT-based SCM (0) Upfront PLX (0)	5	<15%**	
<b>NHL (no significant BM involvement) after 2<sup>nd</sup> salvage treatment (of which one at risk), trilinear cytopenias, &gt; 60y</b>	5	CHT-based SCM (0) No upfront PLX (+2)	7	68%	Start apheresis with lower CD34+ threshold
		CHT-based SCM (0) Upfront PLX (0)	5	<15%**	

MM: Multiple Myeloma; NHL: Non-Hodgkin Lymphoma; HL: Hodgkin Lymphoma; SCM: Stem-cell mobilization; BM: Bone marrow; PLX: Plerixafor. \* Either Hb<13 g/dl or Plt<170.000/mmc and WBC<5000/mmc. \*\* Concurrent use of upfront Plx and chemo-based SCT was not frequent in our sample and thus the reported probability may be inaccurate