RESPONSE OF STEROID-REFRACTORY CHRONIC GRAFT VERSUS HOST DISEASE TO EXTRACORPOREAL PHOTOPHERESIS CORRELATES WITH THE DOSE OF CD3+ CD4+ LYMPHOCYTES HARVESTED DURING EARLY TREATMENT CYCLES

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Clinical background

- Extracorporeal photopheresis (ECP) is currently used as second-line therapy for chronic graft versus host disease (cGVHD).
- Retrospective and prospective studies have shown efficacy in steroid refractory/ dependent patients.
- A steroid sparing effect is observed

National Institutes of Health Consensus Development Project on Criteria for Clinical Trials in Chronic Graft-versus-Host Disease: I. Diagnosis and Staging Working Group Report

Biology of Blood and Marrow Transplantation 11:945-955 (2005)

1083-8791/05/1112-0002$30.00/0
doi:10.1016/j.bbmt.2005.09.004

Measuring Therapeutic Response in Chronic Graft-versus-Host Disease: National Institutes of Health Consensus Development Project on Criteria for Clinical Trials in Chronic Graft-versus-Host Disease: IV. Response Criteria Working Group Report

Biology of Blood and Marrow Transplantation 12:252-266 (2006)

1083-8791/06/1203-0002$32.00/0
doi:10.1016/j.bbmt.2006.01.008

Prognostic factors

(Patient related)

- **Karnofsky score**
- **Lower steroid dose at ECP initiation and during 1st month of treatment**
- **GVHD subtype**

Other prognostic factors

Patient related:

- **Early increase in the peripheral blood of regulatory T-cells**

Treatment related:

- **Dose of nucleated cells, collected and infused during ECP**

However, whether doses of lymphocyte subpopulations harvested correlate with clinical response is largely undetermined

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(I) SELECTION CRITERIA

- Chronic GVHD treated with ECP (two consecutive procedures twice monthly until partial response (PR), thereafter monthly)
- Availability of complete FCM data on each ECP product
- ECP treatment lasting for at least three months

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(I) IMMUNOPHENOTYPE ANALYSIS

Absolute counts of lymphocytes and their subpopulations on each ECP product (single-platform technology)

CD3FITC/CD16-56PE/CD45PerCP-Cy5.5/CD4PE-Cy7/CD19APC/CD8APCCy7

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(III) STATISTICAL ANALYSIS

- As possible predictive factors for PR, the following FCM data were tested (log-rank test):
- For each cell population,
  - (i) the mean dose harvested per ECP during the first 3 months
  - (ii) the cumulative dose harvested during the first 3 months
  - (iii) the cumulative dose harvested until attainment of PR or last follow-up.
- If significant P value, ROC curves of sensitivity and specificity to detect the most accurate predictive

Patients (n = 12)

Median Age, Yrs (Range)	**48 (25-66)**
Males, % of Pts	**50**
Females, % of Pts	**50**
Diagnosis, % of Pts	
Acute Leukemia	***66.6***
Chronic Lymphocytic Leukemia	***8.3***
Myelodysplastic Syndrome	***8.3***
Idiopatic Myelofibrosis	***8.3***
Multiple Myeloma	***8.3***
Median Time Transplant-ECP, Months (Range)	**29 (7-199)**
Organs Involved by GVHD, % of Pts	
Skin	***100***
Mouth	***25.0***
Eyes	***25.0***
Joints	***25.0***
Liver	***9.1***
Gut	***9.1***
Response to ECP, % of Pts	
Patial at 3 Months	***27.3***
Patial at 6 Months	***36.3***
Patial at 9 Months	***8.3***
None	***25.0***

Apheretic yields

Variable	CD3+ x 10^6/Kg		CD3+CD4+ x 10^6/Kg		CD3+CD8+ x 10^6/Kg		CD56+ x 10^6/Kg		CD19+ x 10^6/Kg	
	Median	Range	Median	Range	Median	Range	Median	Range	Median	Range
Mean Single Dose during the 1st 3 Mo	45	14-166	19,57	5-66	23,91	7-92	10,6	1-22	17	4-128
Cumulative Dose during the 1st 3 Mo	485	154-1811	216	65-688	205	57-1012	122	14-219	174,5	44-1409
Cumulative Dose at PR/Last FU	1010,5	397-2298	404,5	234-843	506	110-1299	209	74-796	237	132-1678

Statistical analysis

Log Rank P value

	GB (x 10^6/kg)	Linfociti (x 10^6/kg)	CD3+ (x 10^6/kg)	CD3+CD4+ (x 10^6/kg)	CD3+CD8+ (x 10^6/kg)	CD16/56+ (x 10^6/kg)	CD19/Kg (x 10^6/kg)
Mean Single Dose during the 1st 3 Mo	0,09	0,11	0,11	0,04	0,75	0,37	0,66
Cumulative Dose during the 1st 3 Mo	0,05	0,66	0,11	0,04	0,91	0,35	0,66
Cumulative Dose at PR/Last FU	0,5	0,37	0,5	0,35	0,93	0,50	0,91

Variable	CD3+/Kg		CD3+CD4+/Kg		CD3+CD8+/Kg		CD56+/Kg		CD19/Kg	
	Median	Range	Median	Range	Median	Range	Median	Range	Median	Range
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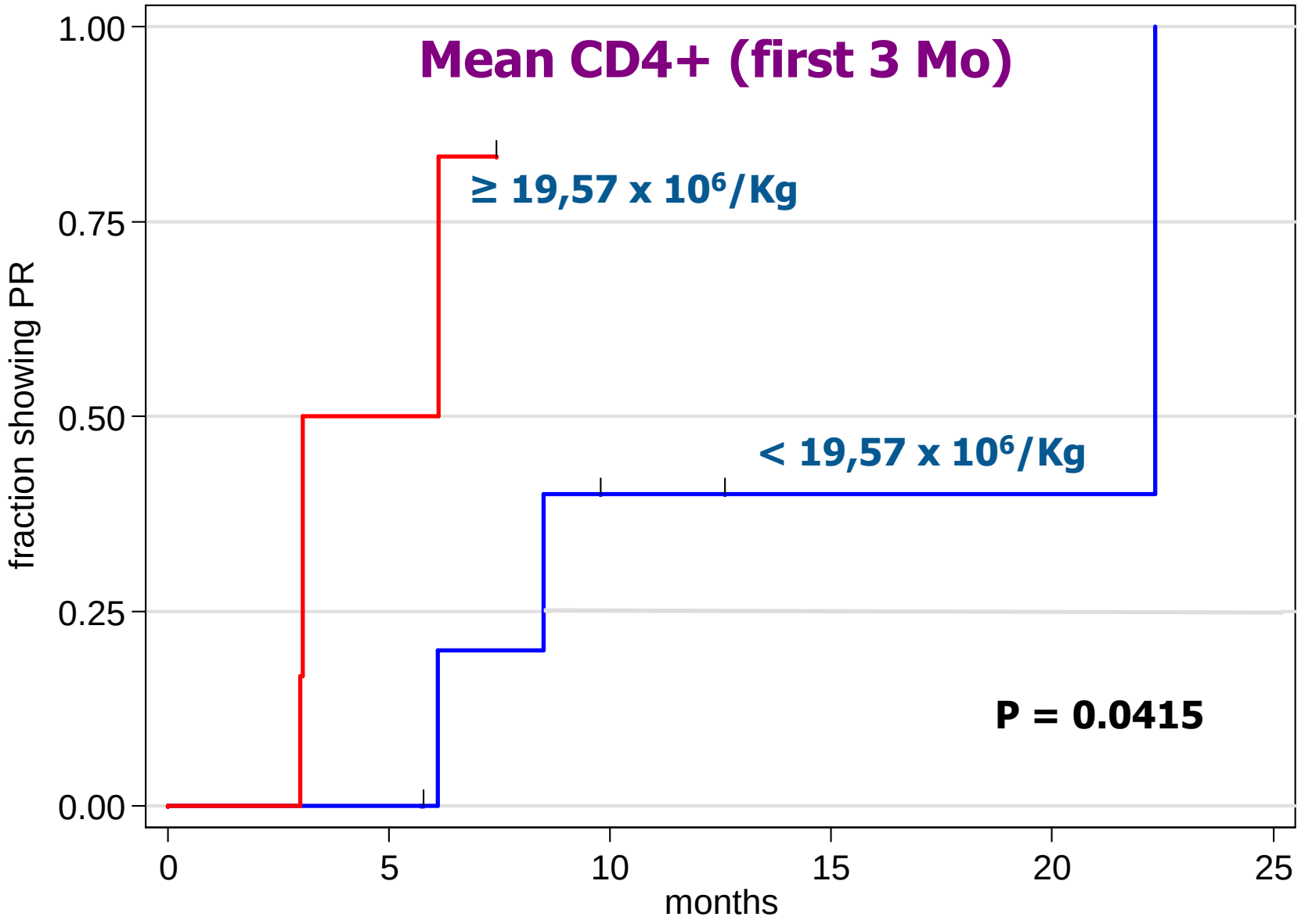
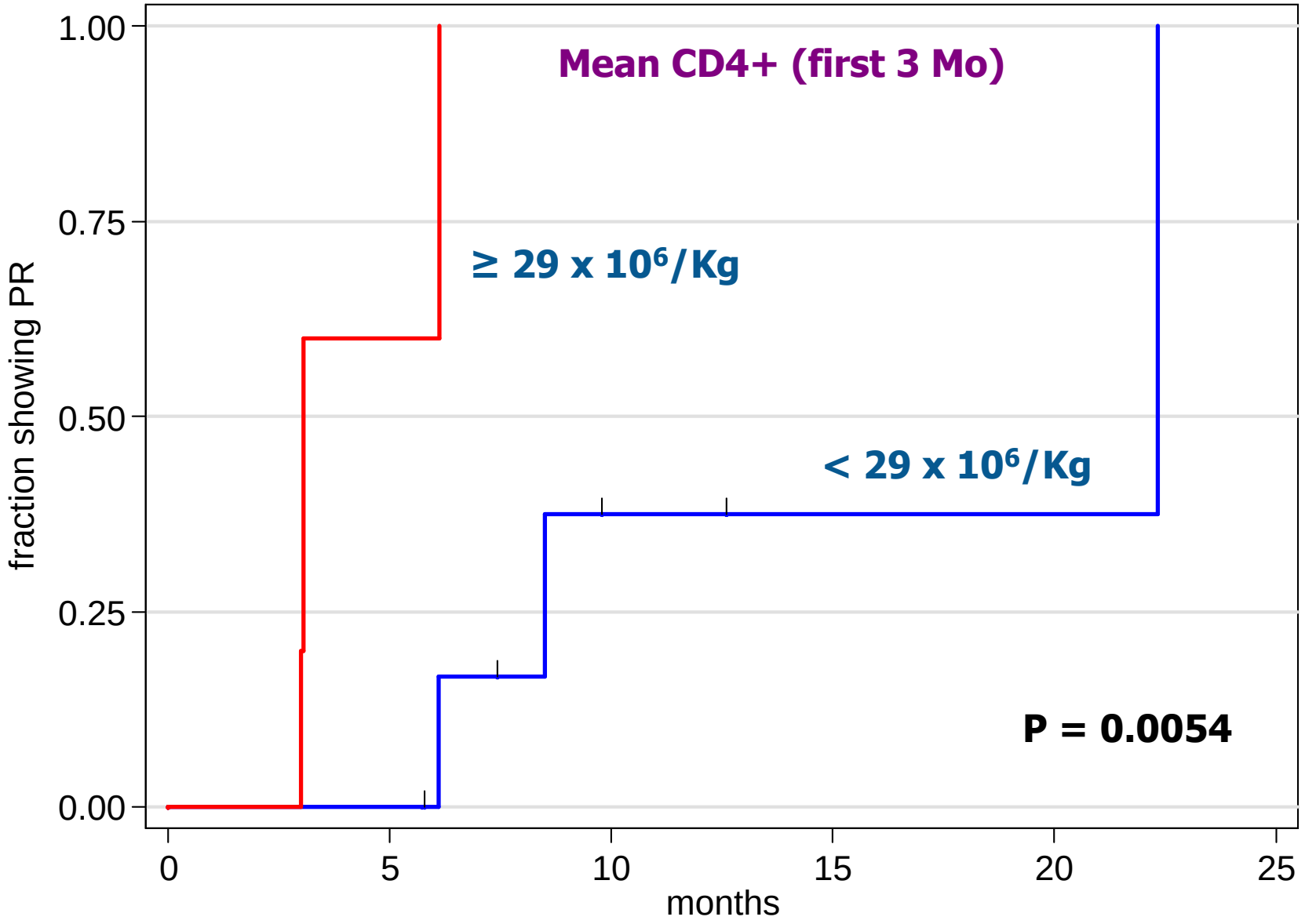

Mean CD4+ (first 3 Mo)
≥ 19,57 x 10⁶/Kg
< 19,57 x 10⁶/Kg
P = 0.0415
fraction showing PR
1.00
0.75
0.50
0.25
0.00
0
5
10
15
20
25
months

29 x 10^6/kg most accurate cut-off for Mean CD3+CD4+ Single Dose during the 1st 3 Mo

Sensitivity	62,5%
Specificity	100%
ROC area	.813
PPV	100%
NPV	57,1%


Mean CD4+ (first 3 Mo)
≥ 29 x 10⁶/Kg
< 29 x 10⁶/Kg
P = 0.0054
fraction showing PR
1.00
0.75
0.50
0.25
0.00
0
5
10
15
20
25
months

Log Rank P value

	GB (x 10^6/kg)	Linfociti (x 10^6/kg)	CD3+ (x 10^6/kg)	CD3+CD4+ (x 10^6/kg)	CD3+CD8+ (x 10^6/kg)	CD16/56+ (x 10^6/kg)	CD19/Kg (x 10^6/kg)
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Cumulative Dose during the 1st 3 Mo	0,05	0,66	0,11	0,04	0,91	0,35	0,66
Cumulative Dose at PR/Last FU	NE	0,37	0,5	0,35	0,93	0,50	0,91

Variable	CD3+/Kg		CD3+CD4+/Kg		CD3+CD8+/Kg		CD56+/Kg		CD19/Kg	
	Median	Range	Median	Range	Median	Range	Median	Range	Median	Range
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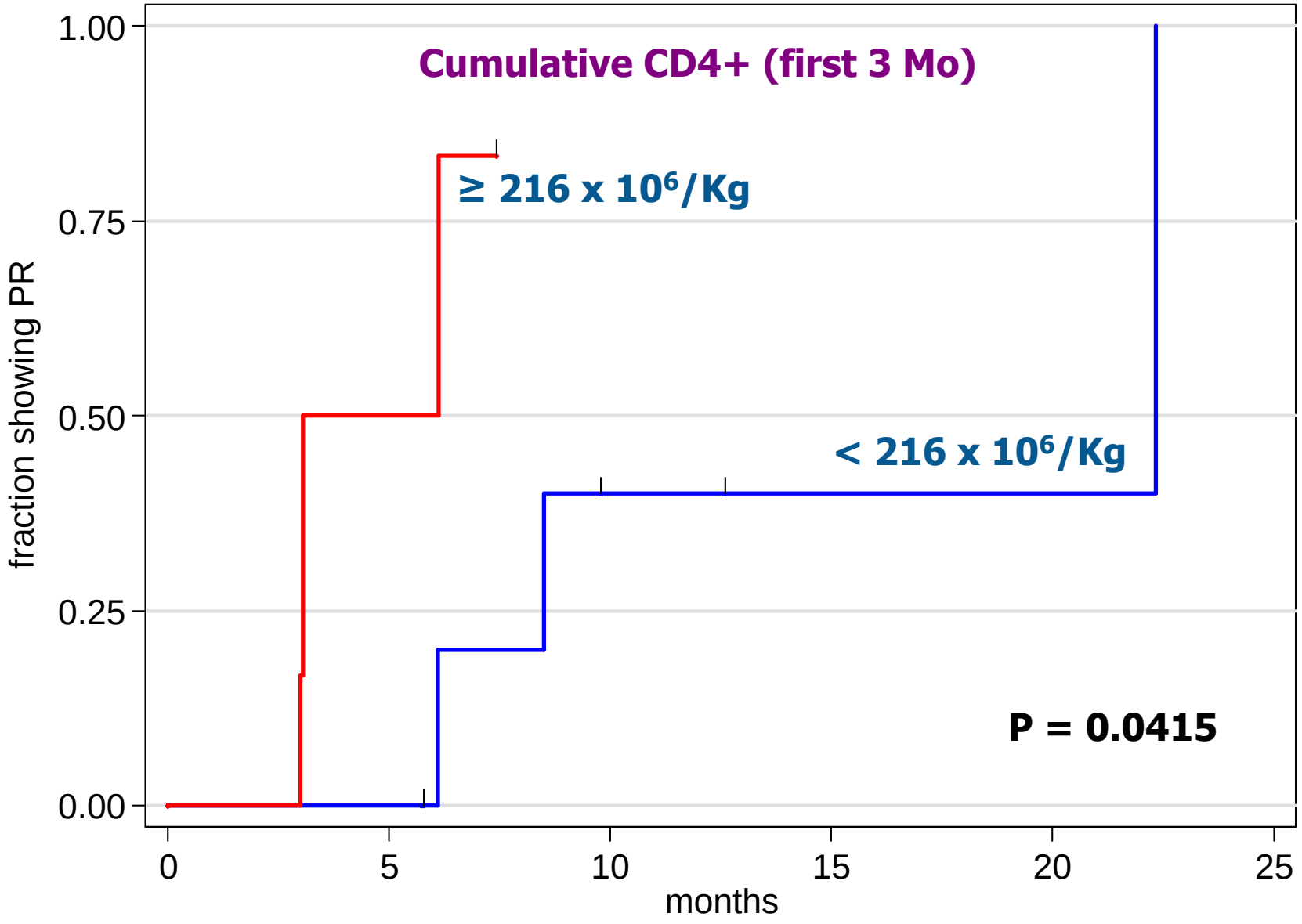
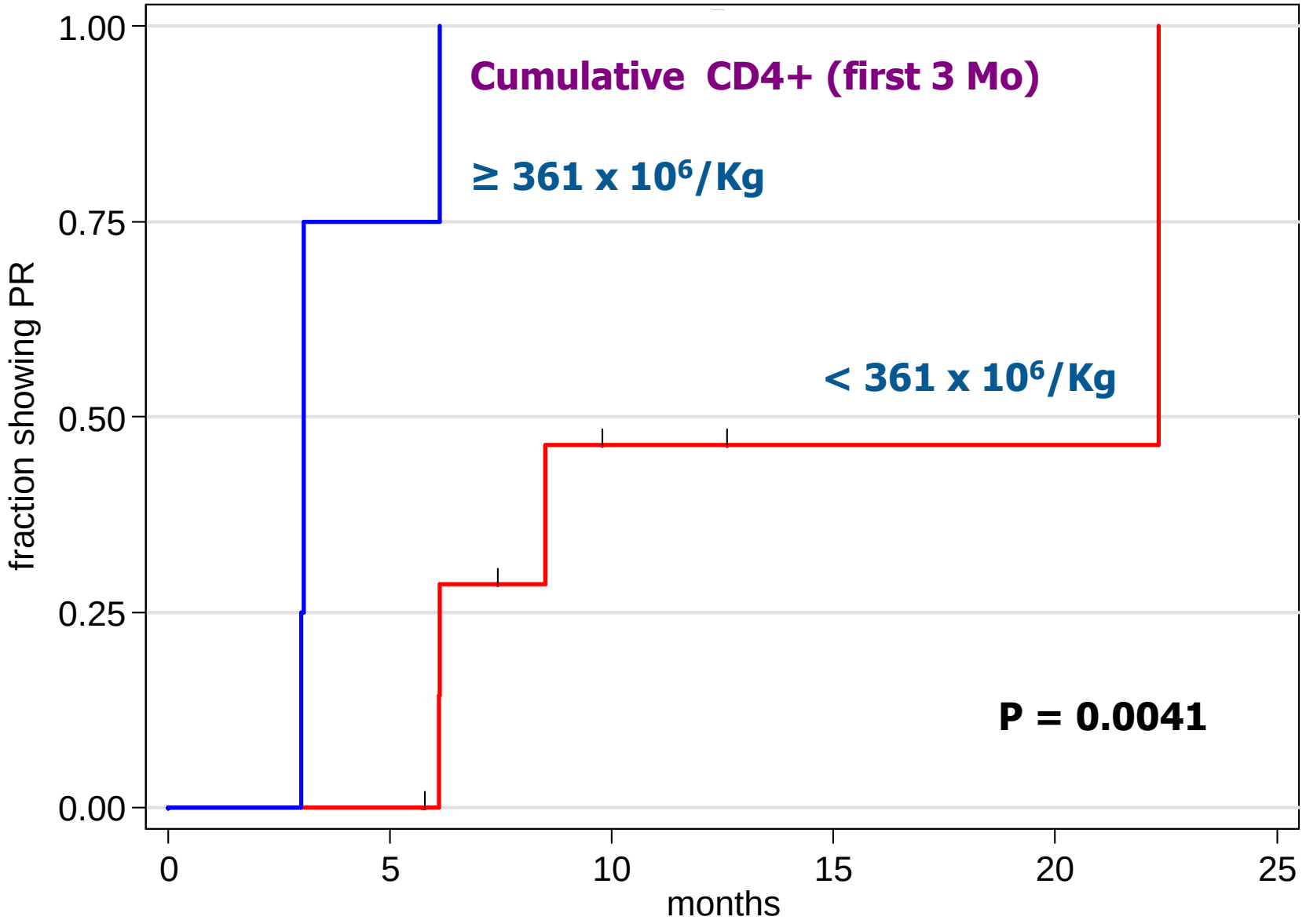

Cumulative CD4+ (first 3 Mo)
≥ 216 x 10⁶/Kg
< 216 x 10⁶/Kg
P = 0.0415
fraction showing PR
1.00
0.75
0.50
0.25
0.00
0
5
10
15
20
25
months

361×10^6/kg as most accurate cut-off for Cumulative CD3+CD4+ Dose during the 1st 3 Mo

Sensitivity	50%
Specificity	100%
ROC area	.75
PPV	100%
NPV	50%


Cumulative CD4+ (first 3 Mo)
≥ 361 x 10⁶/Kg
< 361 x 10⁶/Kg
P = 0.0041
fraction showing PR
1.00
0.75
0.50
0.25
0.00
0
5
10
15
20
25
months

Conclusions

- **CD3+CD4+ cell evaluation in ECP could early predict PR.**
- **In view of the well known lymphocytolytic effect of corticosteroids, the negative impact of dosage/duration of previous immunosuppression on ECP efficacy could be explained.**

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