

**Macrocytosis and dysplastic anemia is associated with the cyclin-dependent kinase 4/6 inhibitor palbociclib in metastatic breast cancer**

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TITLE: Macrocytosis and Dysplastic Anemia is Associated with the CDK 4/6 Inhibitor  
Palbociclib in Metastatic Breast Cancer

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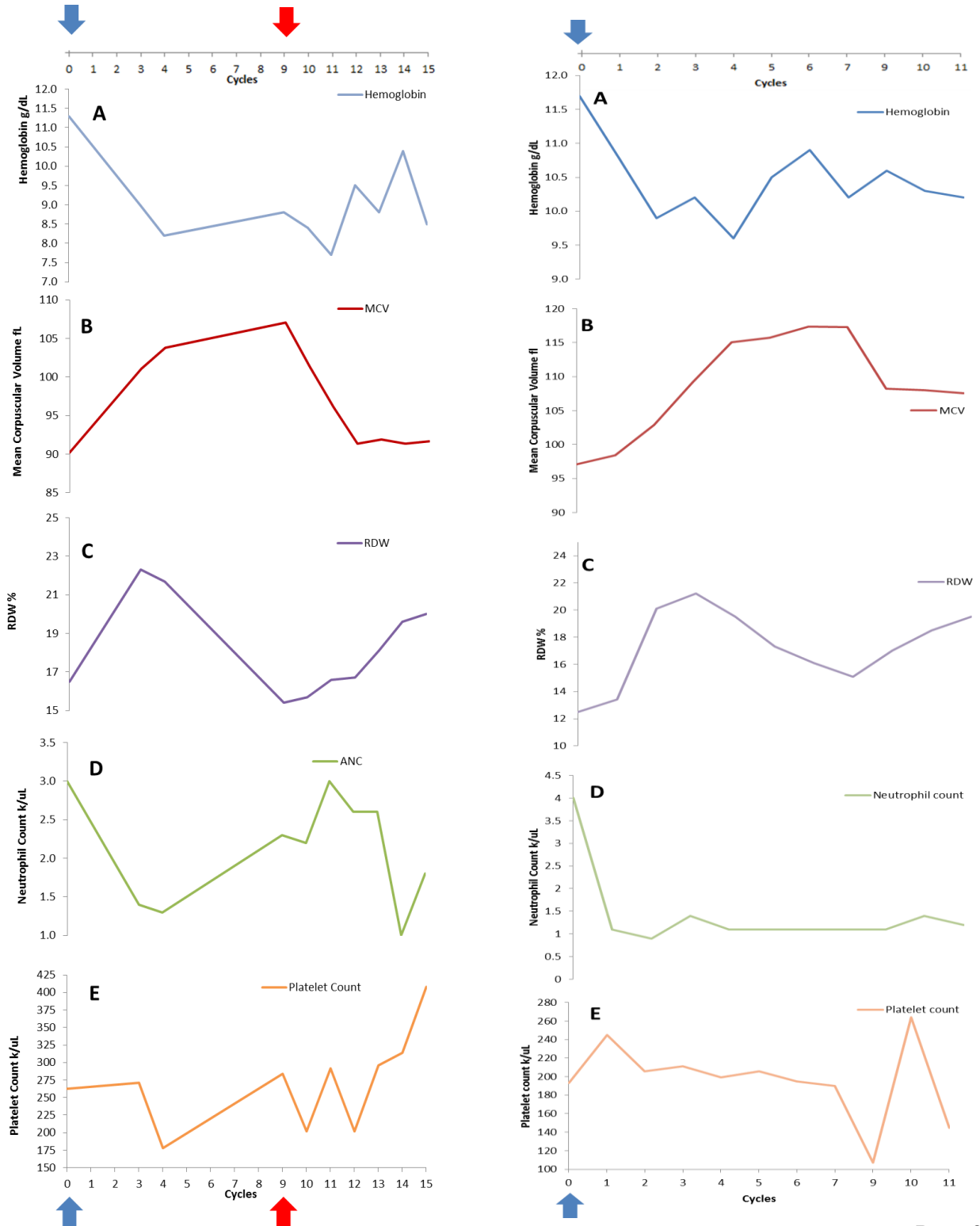
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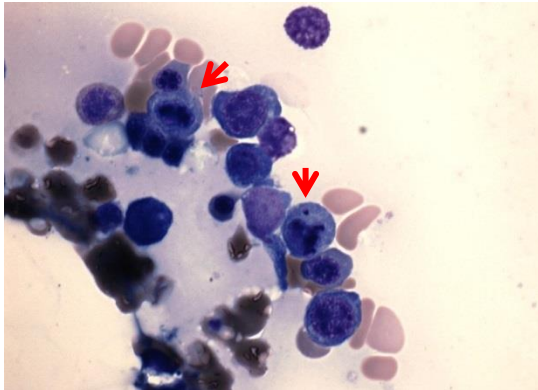
Supplemental Figures

Figure S1

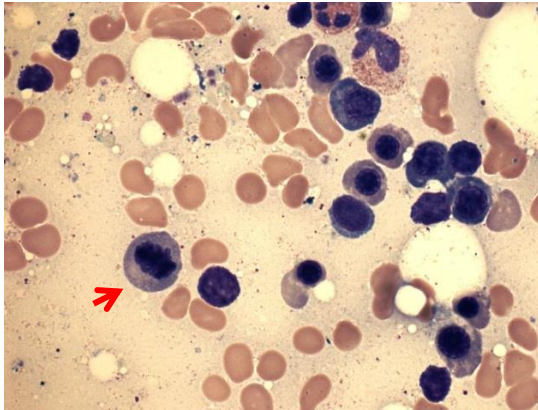


**Figure S1. Hematological parameters with palbociclib use in clinical cases.** Case2 (left figure) already stopped palbociclib. Case3 (right figure) is still on palbociclib treatment. Blue arrow: palbociclib start, Red arrow: Last date of palbociclib treatment. (A) Hemoglobin decreases with palbociclib use. (B) Mean corpuscular volume increases with palbociclib (plateau after 4 months and correlates with lower hemoglobin level), and returns to baseline 4-5 months after palbociclib discontinuation. (C) Red Cell Distribution width (RDW) initially increases but returns to normal after 4 months of palbociclib initiation. (D) Absolute neutrophil count with slight decrease over time with palbociclib use. (E) Platelet count shows normal values during palbociclib treatment.

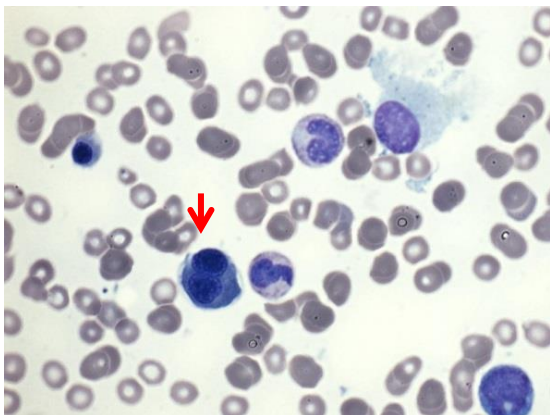
Figure S2.



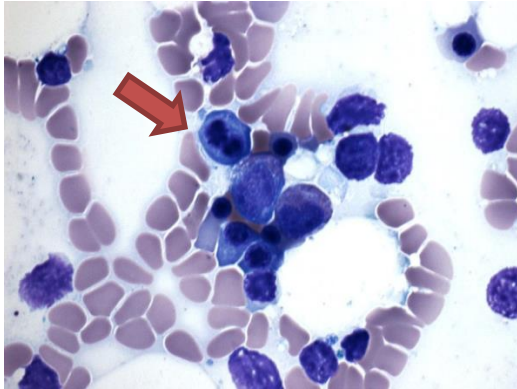
Case 3 Erythroid precursors with nuclear irregularity and karyorrhexis in the bone marrow (arrow) Wright-Giemsa, 100x



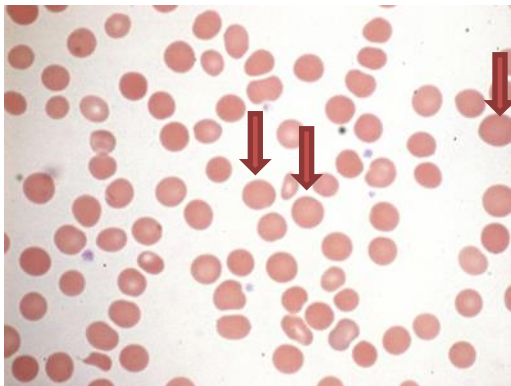
Case 3 Erythroid precursor with nuclear irregularity in the bone marrow (arrow) Wright-Giemsa, 100x



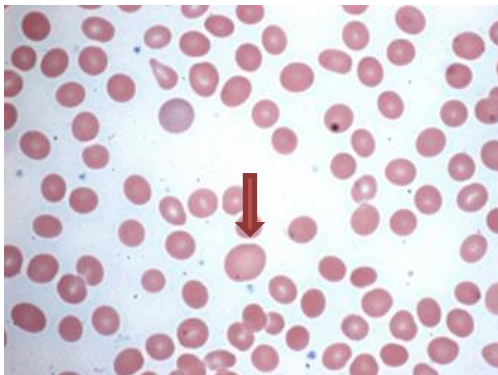
Case 1 Erythroid precursor with binucleation in the bone marrow (arrow) Wright-Giemsa, 100x



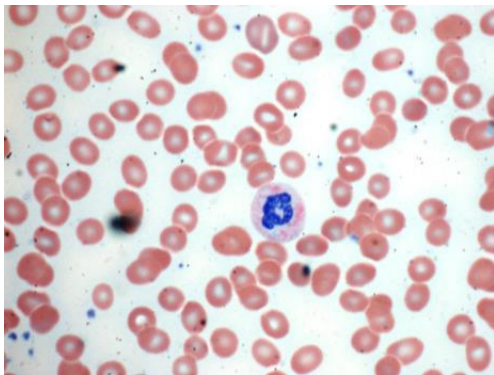
Case 3 Erythroid precursor with karyorrhexis in the bone marrow (arrow) Wright-Giemsa,100x



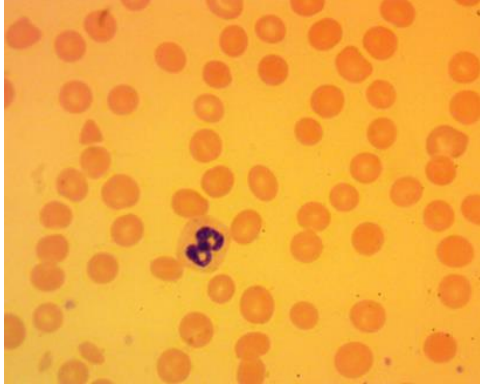
Case 2 Macroovalocytes (arrows) in peripheral blood Wright-Giemsa,40x



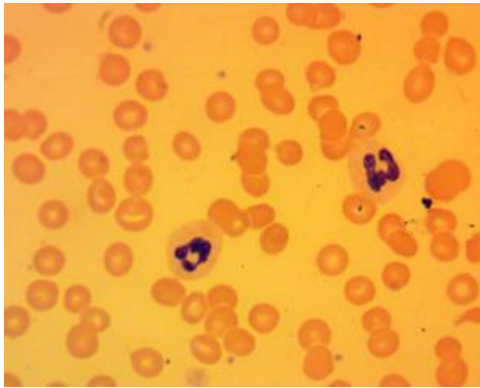
Case 1 Macroovalocyte (arrow) and spherocytosis in peripheral blood Wright-Giemsa, 40x



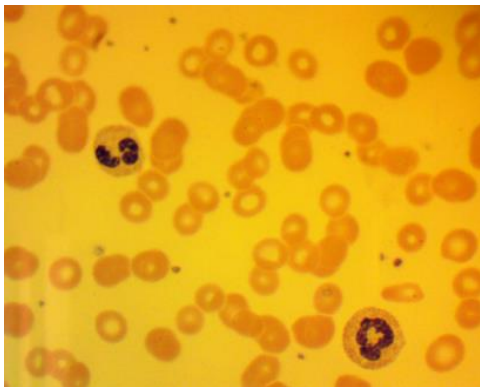
Case 3 dysplastic neutrophil in peripheral blood showing ring formed nucleus and cytoplasmic hypogranules. Wright-Giemsa, 40x



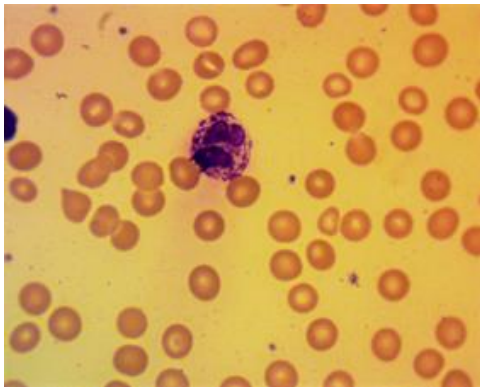
Case 2 dysplastic hypogranular  
neutrophil with double ring formed  
nucleus in peripheral blood  
Wright-Giemsa, 40x



Case 2 dysplastic hypogranular  
neutrophils, one with ring form nucleus  
in peripheral blood Wright-Giemsa, 40x

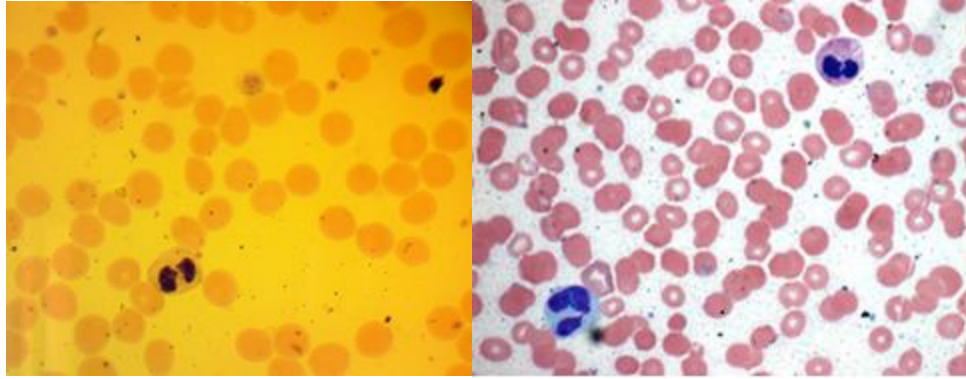


Case 2 bilobed neutrophil and  
dysplastic neutrophil in peripheral  
blood Wright-Giemsa, 40x

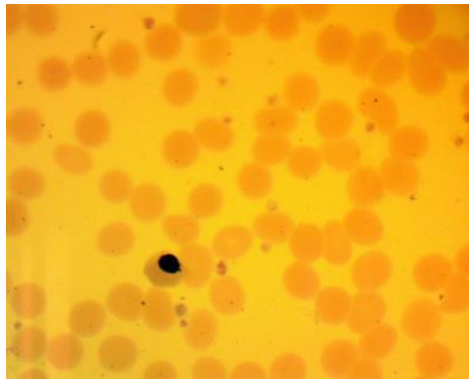


Case 2 Bilobed neutrophil with dysplastic  
granules in peripheral blood Wright-Giemsa,

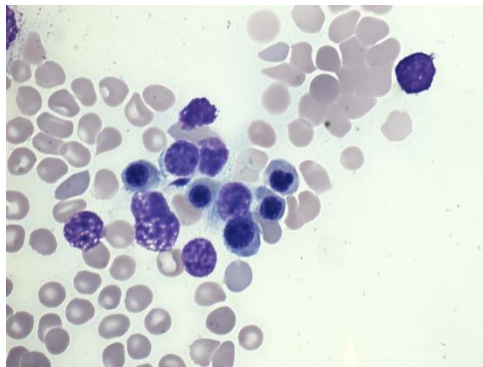




Case 1 Pseudo Pelger  
Huet nucleus/bilobed  
and hypogranular  
neutrophil in peripheral  
blood Wright-Giemsa.



Case 1 nucleated red blood cell in  
peripheral blood Wright-Giemsa, 40x



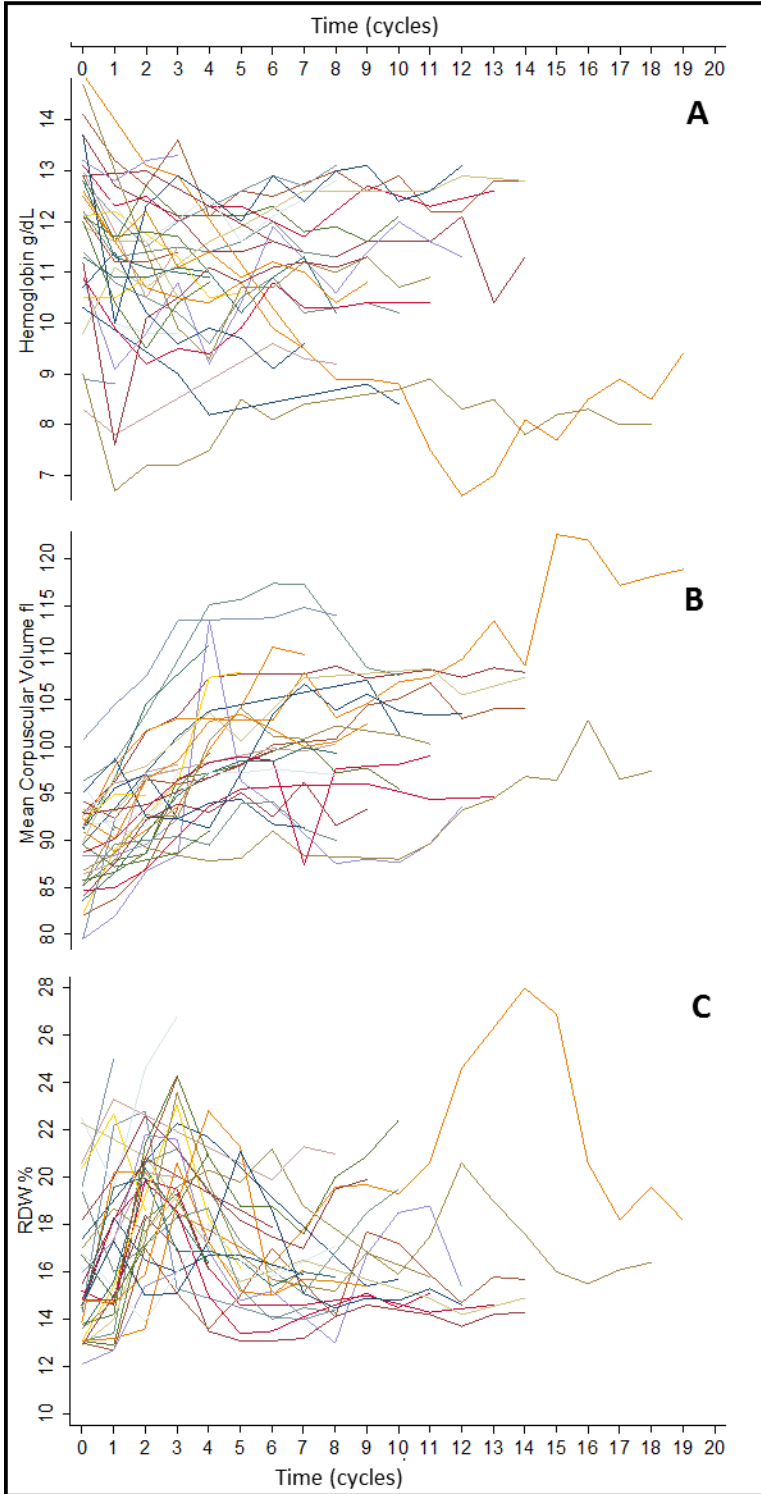
Case 1 Erythroid precursor in the bone  
marrow with nuclear budding 100X

**Figure S2. Bone marrow and peripheral blood smears showing dysplastic changes in erythroid lineage and granulocytes with palbociclib use on three clinical cases.**

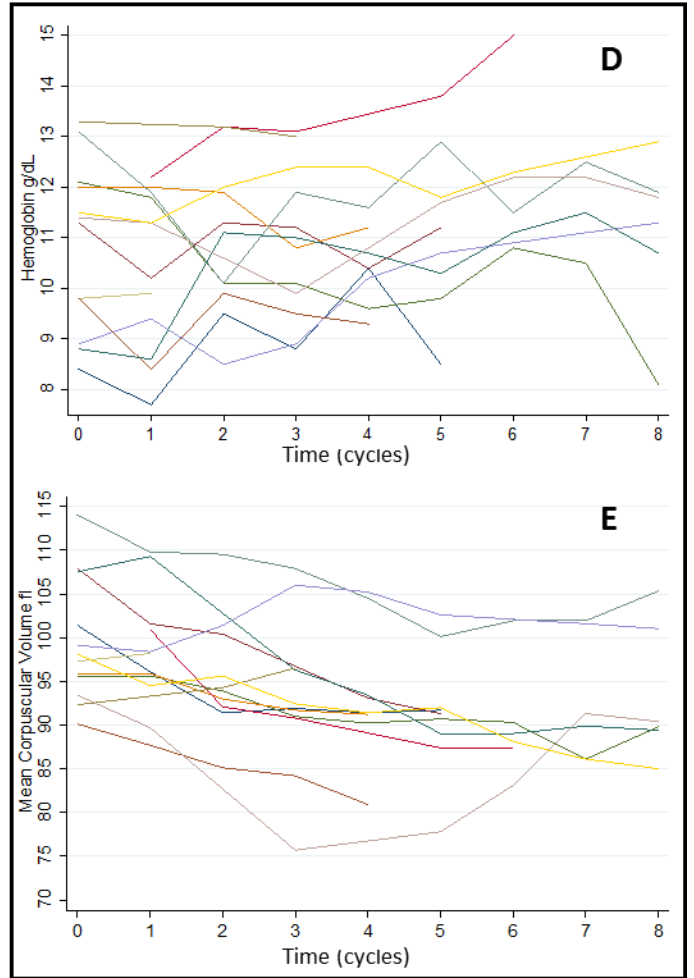


**Figure S3.**

**During Palbociclib Treatment**



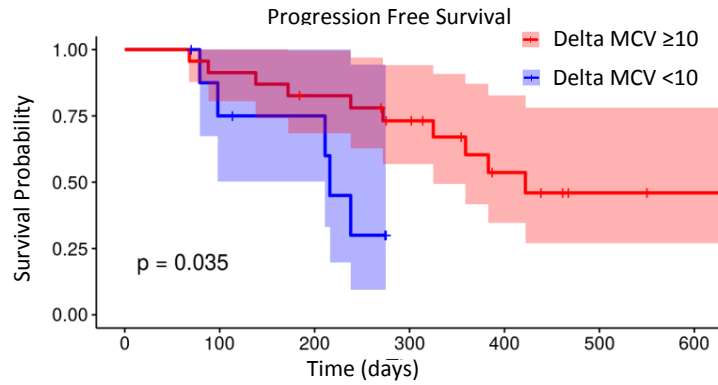
**Post Palbociclib Treatment**



**A-C. Individual change in hematological parameters over time with palbociclib use in retrospective cohort (n=34).** Time 0 is palbociclib start, data censored upon palbociclib discontinuation. (A) Spaghetti plot of individual hemoglobin (Hb) pattern over time. Overall Hb trends to decrease over time after starting palbociclib. (B) Spaghetti plot of individual mean corpuscular volume (MCV) pattern over time. Overall MCV trends to increase over time after starting palbociclib. (C) Spaghetti plot of individual red cell distribution width (RDW) pattern over time. Overall RDW trends to increase right after palbociclib start, returning to baseline after 4-5 months.

**D-E. Hematological parameters for patients who discontinued palbociclib (n=14).** Time 0 is date of palbociclib discontinuation. Individual trend of hemoglobin (Hb) and mean corpuscular volume (MCV) over time. Hb starts to recover after 4-5 months from palbociclib discontinuation. MCV decreases after palbociclib discontinuation and plateaued after 4-5 months from palbociclib discontinuation.

**Figure S4**



**Figure S4. Progression-free-survival for patients receiving palbociclib for advanced breast cancer. (n=32).** Delta MCV $\geq$  10 fl was achieved for 23 patients. Delta MCV< 10 fl was present in 9 patients. Median progression-free-survival (PFS) for patients with delta MCV $\geq$ 10 fl was 422 days compared to 216 days for those with delta MCV<10 fl (HR 0.29,  $p=0.035$ ); however, when delta MCV was treated as a time-dependent variable this difference was not statistically significant (HR 0.59,  $p=0.4$ ).

## Supplemental Tables

**Table S1.**

Parameter	Normal range	Case1	Case2	Case3
Age (years)		68	69	71
Palbociclib duration(months)		14	9	7
RBC (MIL/uL )	4.0-5.2	3.1	5.1	2.63
Hemoglobin (g/dL)	12.3-15.3	10.4	8.8	10.2
MCV (fl)	80-96	107.9	107.1	117.3
RDW (%)	12.4-16.4	14.3	15.4	15.1
MCH (pg)	27-33	36.4	34.9	38.8
TSH (uU/mL )	0.4-4.6	4.37	3.02	1.85
Erythropoietin level (mU/mL )	4.1-19.5	28.8	76.7	
Folate level (ng/mL )	3-17.5	>20	11.5	>20
B12 level (pg/mL )	211-946	920	437	1009
Methylmalonic acid (nmol/L )	87-318	132	152	
Reticulocyte %	0.8-2.2	1.4		1.3
Reticulocyte count (k/uL)		39.2		31.6
Ferritin (ng/ml )	10-150	305	1971	180

Iron level ( ug/dL )	65-175	39	71	82
Iron saturation (%)	26-43	13	30	24
Total Iron Binding Capacity (ug/dL)	250-410	291	238	339
Soluble transferrin receptor (mg/L )	0.76-1.76	1.39		1.08
Transferrin (mg/dL)	204-360	233	190	271
Intrinsic Factor Antibodies		Negative	Negative	
Glucose-6-phosphate dehydrogenase screen		Negative	Negative	
Hepatitis B surface antigen		Non-reactive		Non-reactive
Hepatitis C antibodies		Non-reactive		Non-reactive
SPEP		No monoclonal paraproteinemia		No monoclonal paraproteinemia

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**Table S1. Laboratory characteristics of three clinical cases treated with palbociclib and developed dysplastic, macrocytic anemia.**

RBC: Red blood cell count. MCV: Mean corpuscular volume. MCH: Mean corpuscular hemoglobin. RDW: Red cell distribution width. TSH: Thyroid stimulating hormone. SPEP: Serum protein electrophoresis.

**Table S2**

Variable	Results
Palbociclib cycles, median (range)	8 (1-19)
Age, median years (IQR)	65 (57-74)
Menopausal status (%)	
Pre-menopausal	3 (9)
Peri-menopausal	1 (3)
Post-menopausal	27 (79)
Visceral involvement (%)	
Yes	19 (56)
No	13 (38)
Uncertain	2 (6)
Number of patients with prior treatments (%)	
Chemotherapy	12 (35)
Endocrine therapy	22 (65)
No chemotherapy or endocrine therapy	9 (26)
Endocrine therapy combined with palbociclib (%)	
Fulvestrant	11 (32)
Letrozole	23 (68)
Mean Corpuscular Volume (fl), median(IQR) <sup>1</sup>	
Baseline MCV	89.8 (85.2-92.6)
Maximum MCV during palbociclib	100.1 (97.1-107.9)
Delta MCV <sup>2</sup>	12.4 (7.7-17.5)
Macrocytosis during palbociclib (%) <sup>3</sup>	16 (47)
Hemoglobin (g/dL), median (IQR)	
Baseline Hb	12.1(10.9-13.0)
Hb during palbociclib	10.4 (9.2-11.2)
Median decrease in Hb	1.6 (1.1-2.3)

**Table S2. Baseline characteristics of retrospective cohort study of patients treated with palbociclib for advanced breast cancer (n=34).** IQR: interquartile range. MCV: mean corpuscular volume. Hb: hemoglobin. (1) Median baseline MCV (89.78 fl) vs. median highest MCV (100.1 fl) ( $p<0.0001$ ). (2) Delta MCV: maximum MCV with palbociclib – baseline MCV. (3) Macrocytosis:  $MCV \geq 100$ . One patient had macrocytosis before palbociclib.



**Table S3**

Variable	Delta MCV < 10 fl (N=10)	Delta MCV ≥10 fl (N=24)	P value
Palbociclib cycles, median (IQR)	5(3-8)	10(6-13)	0.006
Endocrine therapy combined with palbociclib (%)			0.54
Fulvestrant	4(36.4)	7(64.6)	
Letrozole	6(26.1)	11(73.9)	
Age, median (IQR)	67(57-74)	62(56-75)	0.84
Decrease in hemoglobin (g/dL), median (IQR)	1.0 (0.5-1.4)	1.95(1.4-2.8)	0.001
Hispanic (%)			0.44
No	5(41.7)	7(58.3)	
Yes	2(25.0)	6(75.0)	
Tumor grade- differentiation (%)			0.32
Well	0(0.0)	3(100.0)	
Moderate	5(41.7)	7(58.3)	
Poorly	1(20.0)	4(80.0)	
Visceral involvement (%)			0.47
No	5(38.5)	8(61.5)	
Yes	5(26.3)	14(73.3)	
Adjuvant/neoadjuvant chemotherapy (%)			0.78
No	4(30.8)	9(69.2)	
Yes	5(26.3)	14(73.7)	
Palliative chemotherapy (%)			0.008
No	3(14.3)	18(85.7)	
Yes	7(58.3)	5(41.7)	
Palliative endocrine therapy (%)			0.06
No	1(9.1)	10(90.9)	
Yes	9(40.9)	13(59.1)	

**Table S3. Univariate analysis of association between clinico-pathological variables and significant change in mean corpuscular volume in retrospective cohort study of patients treated with palbociclib for advanced breast cancer (n=34).** IQR: interquartile range. MCV: mean corpuscular volume. Significant change in MCV is defined as  $\Delta \text{MCV} \geq 10 \text{ fl}$  with palbociclib use.