Rational management approach to pure red cell aplasia

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Supplementary Table 1. Diagnostic criteria for PRCA

Severe anemia[$] with	Absolute reticulocyte count < 10,000/µl (Reticulocyte percentage <1%)
	< 0.5% mature erythroblasts in the bone marrow
	Normocellular bone marrow in most cases
	Usually normal cytogenetics*

[$] Hemoglobin < 8 gm/dl; * presence of abnormal cytogenetics indicates myelodysplastic variant of PRCA

Supplementary Table 2. Patient characteristics (n=62)

Age (median in years)*		62 (25 – 87)
Gender (%)	**Male**	34 (55%)
Etiology[#] (%)	**Congenital (DBA)**	4 (7%)
	Acquired	
	· Idiopathic	32 (52%)[$]
	· LGL leukemia	14 (22%)
	· Thymoma	9 (15%)
	· Parvovirus	4 (6%)
Median follow up	40 months (1-133 mo.)	
Total number in remission @ last follow up	46	
OS @ 5 years	0.835 (95% CI 0.695, 1)	
OS @ 10 years	0.674 (95% CI 0.472, 0.963)	
DFS @ 5 years	0.675 (95% CI 0.529, 0.861)	
DFS @ 10 years	0.496 (95% CI 0.321, 0.764)	

*excludes Diamond Blackfan Anemia patients, [#]one patient had both LGL leukemia and positive parvo virus, [$] *P*<.001, comparison among the distribution of PRCA (pure red cell aplasia) between idiopathic, LGL (large granular lymphocytic) leukemia, thymoma and parvovirus related cases

Supplementary Table 3. Definition of responses

Criteria	CR (complete response)	PR (partial response)	NR (no response)
Retic response	Appropriate rise in reticulocyte count after 8 weeks* (reticulocyte responses commensurate to level of hemoglobin rise)	No appropriate rise in reticulocyte count after 8 weeks* of initiating treatment	No appropriate rise in reticulocyte response after 8 weeks* of initiating treatment
Transfusion requirement	Transfusion independence followed by normalization of hemoglobin levels	Still anemic and requiring, but less frequent than before initiating the drug	Anemic and still requiring at the same frequency as before starting the drug

***** This is the earliest time for assessment of response. Response was again re-assessed by 3 months milestone and in less common circumstances monitored for 6 months until calling it as non-responder to a particular treatment.

Supplementary Table 4. List of genes covered by Nextera sequencing

ABL1	DNMT3A	KDM6A	RAD21	U2AF2
APC	ELANE	KDM6B	RAD50	VHL
ASXL1	EP300	KDR	RAD51	VPS45
ASXL2	ETNK1	KIT	RAD51C	WAS
ATM	ETV6	KLF1	RAD51D	WRAP53
ATR	EZH2	KMT2D	RET	WT1
ATRX	FANCA	KRAS	RIT1	XRCC2
BARD1	FANCB	LUC7L2	RPL11	XRCC3
BCOR	FANCC	MFSD11	RPL26	ZRSR2
BCORL1	FANCD2	MLH1	RPL35A	
BRAF	FANCE	MPL	RPL5	
BRCA1	FANCF	MRE11A	RPS10	
BRCA2	FANCG	MSH2	RPS17	
BRCC3	FANCI	MSH6	RPS19	
BRIP1	FANCL	MUTYH	RPS24	
BUB1B	FANCM	MYB88	RPS26	
CALR	FAT4	MYD88	RTEL1	
CBL	FBXW7	NBN	RUNX1	
CBLB	FH	NF1	RUNX1T1	
CBLC	FLT3	NFE2	SAMD9	
CDAN1	G3BP1	NHP2	SAMD9L	
CDC25B	G6PC3	NOP10	SBDS	
CDH1	GALNT12	NOTCH1	SETBP1	
CDK4	GAR1	NPM1	SETD2	
CDKN2A	GATA1	NRAS	SF3A1	
CEBPA	GATA2	NRP1	SF3B1	
CFTR	GFI1	PALB2	SH2B3	
CHEK2	GFI1B	PCLO	SMAD4	
CSF1R	GIGYF1	PDGFRA	SMC1A	
CSF2RA	GNAS	PDS5B	SMC3	
CSF2RB	HAX1	PHF6	SRSF2	
CSF3R	HOXB13	PIGA	STAG2	
CSNK1A1	HRAS	PIGT	STAT3	
CTCF	IDH1	PMS2	STK11	
CUX1	IDH2	POT1	STK4	
DCLRE1C	IKZF1	PRF1	TERT	
DDX41	IRF1	PRPF8	TET2	
DKC1	JAK2	PRSS1	TINF2	
DNAJC21	JAK3	PTEN	TP53	
DNJC21	JARID2	PTPN11	U2AF1	

Supplementary Table 5. **Characteristics of Diamond Blackfan Anemia patients in our cohort**

Characteristics of DBA patients	
Gender	50% males
Family history +	3/4 patients
RPS 19 gene mutation	3/3 patients
HbF @ diagnosis	NA
Median MCV @ last follow up	99.5 fl (normal range 80-96 fl)
Last bone marrow cytogenetics	Normal in 4/4 patients
Disease status @ last follow up	3 CR 1 died from other illness
Steroid responsive	3/4 patients

Supplementary table 6. LGL diagnostic criteria

LGL diagnostic criteria		
Splenomegaly	Plus, one or more of ->	Peripheral LGL count > $0.5x10^9$/l
Neutropenia (ANC<1.5/ k/uL)/Anemia (Hb<11 g/dl)		FCM – Clonal Vβ expansion
Lymphocytosis (ALC > 4.0 k/uL)		NK cell phenotype
Autoimmune conditions		Activated T Cell phenotype
		PB TCR-γ using PCR

ANC - absolute neutrophil count, Hb - hemoglobin, ALC - absolute lymphocyte count

Supplementary Table 7. Mutations identified in different PRCA groups[$] by Nextera sequencing

Sample ID	Diagnosis	Chromosome	Gene	VAF	Transcript	Nucleotide change	AA change
Sample1	Idiopathic	17	PRPF8	44.4%	NM_006445	A623G	Y208C
Sample1	Idiopathic	22	CSF2RB	49.8%	NM_000395	C2105A	T702N
Sample1	Idiopathic	12	KMT2D	51.0%	NM_003482	G12559A	G4187R
Sample1	Idiopathic	1	HAX1	45.3%	NM_001018837	T15A	D5E
Sample2	Idiopathic	8	RUNX1T1	49.6%	NM_001198679	A256G	T86A
Sample3	Idiopathic	17	KDM6B	100.0%	NM_001080424	756_761del	252_254del
Sample3	Idiopathic	11	ATM	49.5%	NM_000051	T6898G	W2300G
Sample3	Idiopathic	3	GATA2	45.7%	NM_001145662	G1270T	A424S
Sample3	Idiopathic	1	VPS45	47.7%	NM_001279355	G532C	E178Q
Sample4	Idiopathic	X	BCOR	46.6%	NM_001123383	G112A	A38T
Sample4	Idiopathic	12	KMT2D	48.6%	NM_003482	C761A	A254D
Sample5	Idiopathic	7	PCLO	46.7%	NM_014510	G2717C	S906T
Sample6	Idiopathic	17	PRPF8	51.6%	NM_006445	T745A	L249M
Sample6	Idiopathic	17	TP53	49.1%	NM_001126115	G65A	G22D
Sample6	Idiopathic	19	KLF1	58.1%	NM_006563	T712A	C238S
Sample7*	LGL	17	KDM6B	100 %	NM_001080424	758_759insACC	P253delinsPP
Sample7	LGL	17	STAT3	13.8%	NM_003150	G1981T	D661Y
Sample7	LGL	12	KMT2D	15.8%	NM_003482	3668_3670del	1223_1224del
Sample7	LGL	20	GNAS	47.2%	NM_016592	57dupC	D19fs
Sample7	LGL	5	APC	53.1%	NM_001127511	G134C	R45P
Sample7	LGL	4	FAT4	20.1%	NM_001291285	C4339T	P1447S
Sample7	LGL	3	ATR	49.5%	NM_001184	A721G	S241G
Sample8*	LGL	17	KDM6B	38.7%	NM_001080424	T752C	L251S
Sample8	LGL	15	CDAN1	54.2%	NM_138477	A799T	T267S
Sample8	LGL	4	PDGFRA	58.3%	NM_006206	T3083C	V1028A
Sample8	LGL	3	GATA2	38.6%	NM_001145662	G1349T	S450I
Sample9	LGL	17	KDM6B	31.8%	NM_001080424	T1417C	C473R
Sample9	LGL	14	TINF2	50.6%	NM_001099274	G928A	A310T
Sample9	LGL	17	STAT3	30.8%	NM_003150	A1840C	S614R
Sample9	LGL	5	NPM1	45.3%	NM_001037738	550_552del	184_184del
Sample10	LGL	7	CUX1	47.9%	NM_001202544	T1897C	C633R
Sample10	LGL	9	NOTCH1	49.2%	NM_017617	G3271A	G1091S
Sample11	LGL	22	EP300	31.8%	NM_001429	3733_3734insGC	V1245fs
Sample12	Parvovirus	17	TP53	46.4%	NM_001126117	T616C	W206R

[$] 1/1 parvo virus, 6/14 in Idiopathic PRCA, 0/5 thymoma cases and 5/9 LGL cases had mutations identified by Nextera sequencing; * also had positive STAT3 by PCR (Supplementary Table 8).

Supplementary Table 8. **STAT3 by PCR in LGL PRCA patients** *

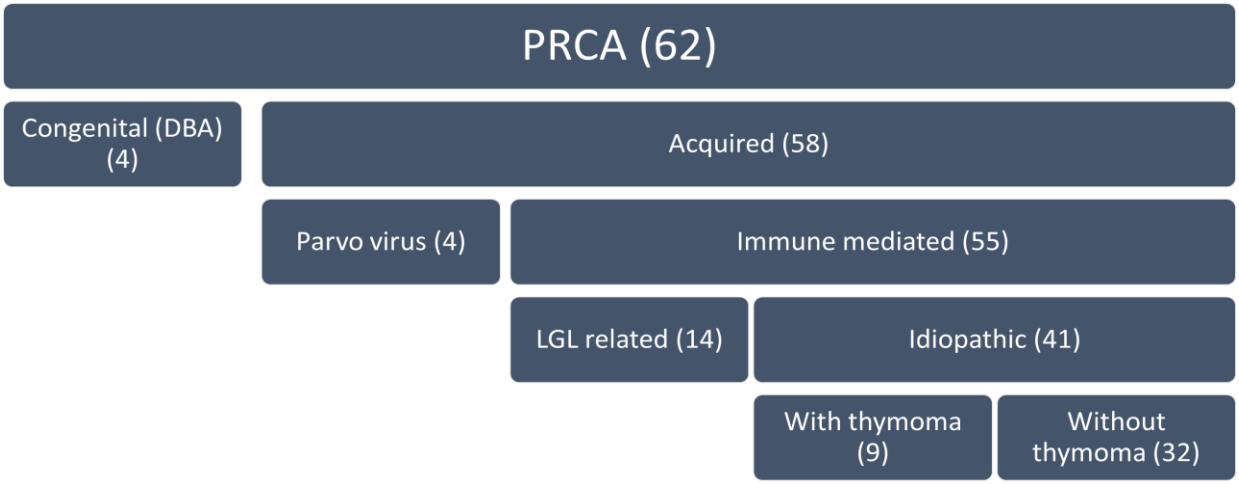
ID / Diagnosis	STAT3 by PCR	Number of mutations	Amino acid change	VAF %
1/ LGL	Yes	2	D661V	32 & 2
2/ LGL	Yes	1	D661V	21
3/ LGL	Yes	1	Y640F	30

* 5/11 LGL PRCA patients had positive STAT3 by PCR (3 listed in this table and 2 listed in Supplementary Table 4)

Supplementary Figure 1. **Clinical classification of PRCA patients (n=62) in our cohort**.

Supplementary Figure 2. **Median CD4/8 and CD 16/56 ratios among different aPRCA groups**. Median CD 4/8 and CD 16/56 ratios were the lowest in the LGL-PRCA followed by thymoma related and idiopathic PRCA.

Supplementary Figure 1



Supplementary Figure 2