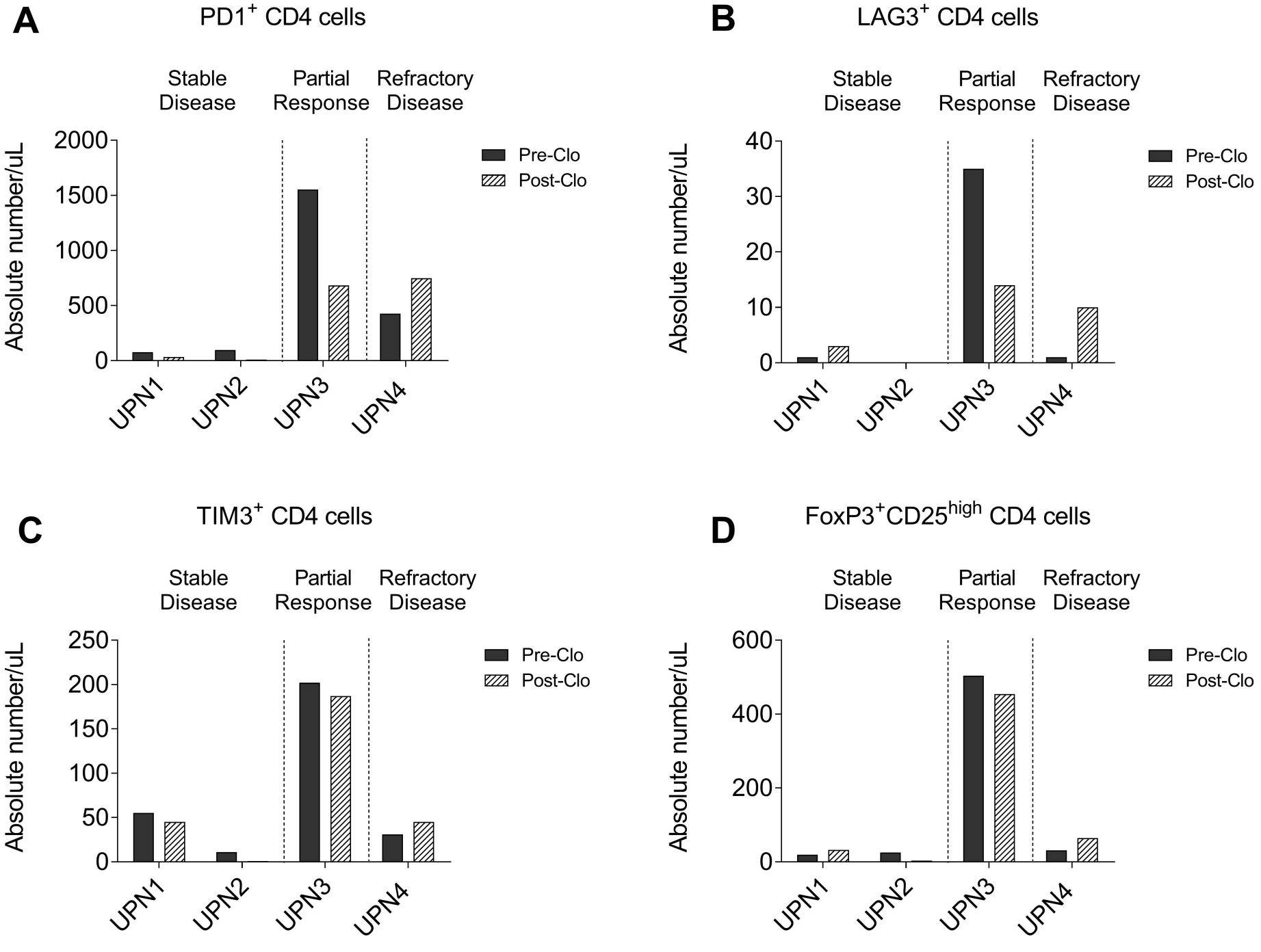
Supplementary material

Clinical study design

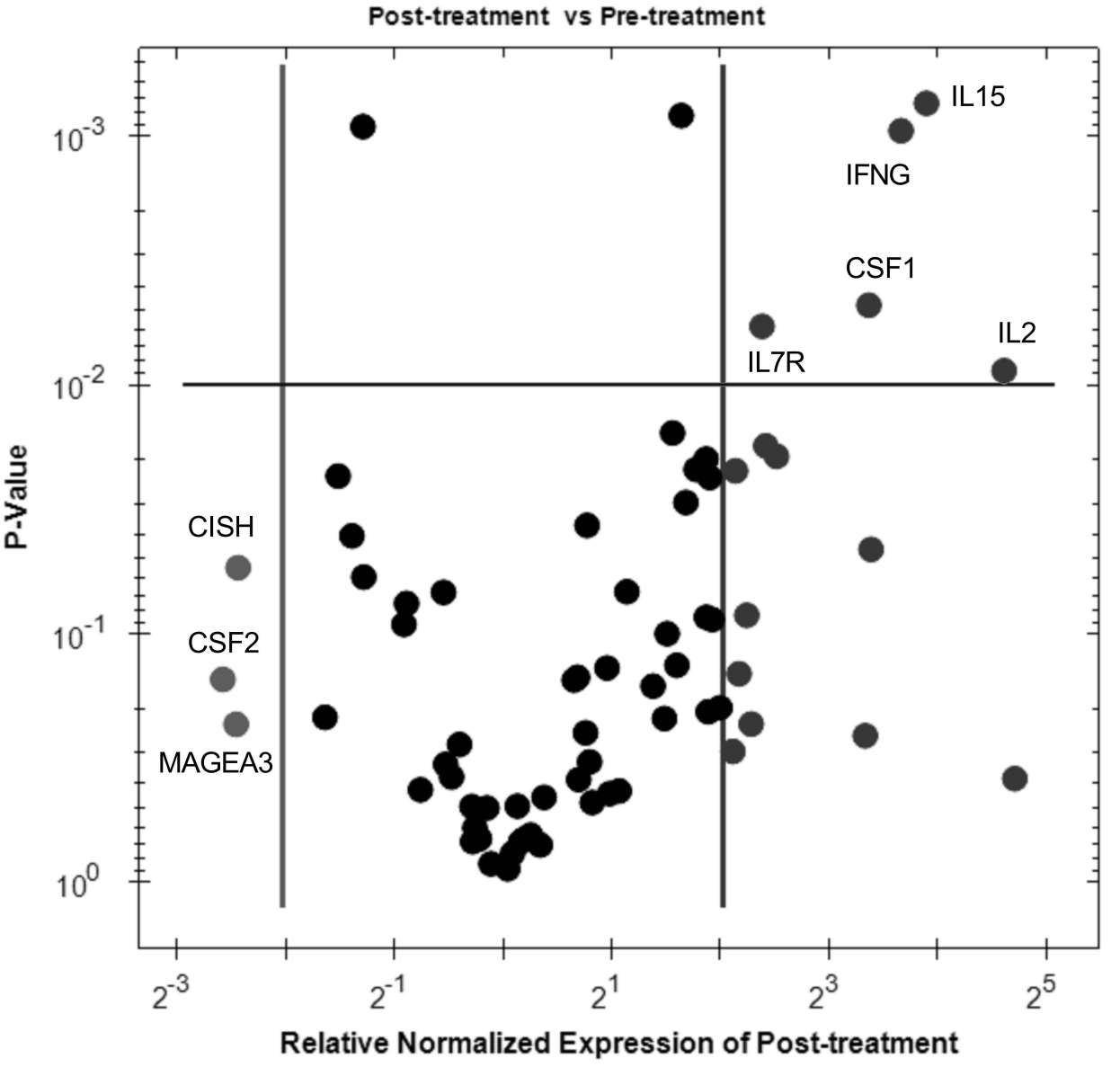
The study was designed as an open label, single institution phase I study at the National Heart, Lung, Blood Institute, National Institutes of Health and was approved by Institutional Review Board (NIH protocol 12-H-0146: NCT01629082). All patients signed an informed consent prior to enrollment and the study was conducted in compliance with the Declaration of Helsinki. The eligibility criteria includes the patients with diagnosis of high risk MDS patients (IPSS risk score at or greater than intermediate 2), CMML, and AML who were not candidates for standard intensive chemotherapy or allogeneic stem cell transplantation. Eligible patients must have failed at least one prior therapy before study enrollment. Patients were excluded if they received clofarabine at any dose or lenalidomide at dose of more than 25mg daily. Inadequate organ functions were also excluded; ejection fraction less than 40% by echocardiogram or MUGA, creatinine clearance less than 60 ml/min, total bilirubin greater than 1.5 times upper limit of normal, aspartate transaminase (AST) or alanine transaminase (ALT) greater than three times upper limit of normal.

Subjects received a single course of intravenous low-dose clofarabine 5mg/m^2/day for five days with pre-medications of ondansetron 16mg by mouth (or 8mg intravenously) and dexamethasone 12m once daily prior to clofarabine infusion. At 28 days after induction therapy with clofarabine, oral lenalidomide was initiated with dose escalation from 25 mg daily up to 50 mg daily for 21 days of 28 days. In the absence of dose limiting toxicity (DLT) or disease progression, subjects received lenalidomide 10 mg daily in 28 day-cycles with dose adjustments, for up to 12 cycles. DLT was defined as at or greater than grade 3 non-hematologic toxicity related to lenalidomide, prolonged bone marrow aplasia, or grade 4 thromboembolic event related to study treatment. The primary study endpoint was the toxicity profile of this novel treatment combination in each cohort. Patients remained on study until one of the following conditions was met: unacceptable toxicity or treating physician discretion or death. Response assessment was performed according to International Working Group (IWG) response criteria.

Supplementary Figure 1



Supplementary Fig 2



Supplementary Table 1.

Antibody	Fluorochrome	Vendor	Cat.No	Clone	Isotype
T cell surface phenotype					
CD4	BUV 396	BD Horizon	563550	SK3	Mouse IgG1,kappa
CD8	BUV496	BD Horizon	564804	RPA-T8	Mouse, IgG1, kappa
CD45RO	BV711	Biolegend	304236	IVN31	Mouse, IgG2a, kappa
CD127	BV650	Biolegend	351326	AO19D5	Mouse, IgG1, kappa
CD3	BV605	Biolegend	317321	OKT3	Mouse, IgG2a, kappa
CD14	PB	Life technologies	MHCD1428	TuK4	Mouse, IgG2a, kappa
CD19	PB	Life technologies	MHCD1928	SJ25-C1	Mouse, IgG1, kappa
CD27	BV510	Biolegend	302836	O323	Mouse, IgG1, kappa
CD25	Alexa Fluor 700	Biolegend	356118	M-A251	Mouse, IgG1 kappa
CD45RA	APCCy7	Biolgend	304128	HI100	Mouse, IgG2b, kappa
PD-1	PE	Biolegend	329906	EH12	Mouse IgG1, kappa
LAG-3	FITC	eBioscience	11-2239-42	3DS223H	Mouse, IgG1, kappa
LAG-3	APC	eBioscience	17-2239-42	3DS223H	Mouse, IgG1, kappa
TIM-3	FITC	Biolegend	345022	F38-2E2	Mouse, IgG1, kappa

PDL1	PE/dazzle	Biolegend	329732	29E.2A3	Mouse, IgG2b, kappa
CD127(IL-7Rα)	BV711	Biolegend	351328	A019D5	Mouse, IgG1, kappa
CD45RA	BV650	Biolegend	304136	HI100	Mouse, IgG2b, kappa
HLADR	APCCy7	Biolegend	307618	L243	Mouse, IgG2a, kappa
CD39	PECy7	Biolegend	328212	A1	Mouse, IgG1, kappa
CD95	PE/Dazzle	Biolegend	305634	DX2	Mouse, IgG1, kappa
CCR7(CD197)	BV785	Biolegend	353230	G043H7	Mouse, IgG2a, kappa
PDL1(CD 274)	PECy7	BD Pharmingen	558017	MIH100	Mouse, IgG1, kappa
Intracellular staining					
FoxP3	APC	eBioscience	17-4777-42	234A/E7	Mouse, IgG1, kappa
Helios	PE	Biolegend	137216	22F6	Armenian Hamster IgG
NK cell phenotype					
CD158a,h	PECy5.5	Beckman Coulter	A66898	EB6B	Mouse, IgG1, kappa

CD158b1/b2,j	PECy5.5	Beckman Coulter	A66900	GL183	Mouse, IgG1, kappa
KIR3DL1	Alexa700	Biolegend	312712	DX-9	Mouse, IgG1, kappa
LIR1	APC	eBioscience	17-5129-42	HP-F1	Mouse, IgG1, kappa
CD56	PECy7	BD	335809	NCAM16.2	Mouse, IgG2b, kappa
NKG2A	PE	Beckman Coulter	IM3291U	z199	Mouse, IgG2b, kappa
CD57	FITC	BD Pharmingen	555619	NK-1	Mouse, IgM, kappa
CD16	V500	BD Horizon	561394	3G8	Mouse, IgG1, kappa

Supplemenatry Figure/Table Legends:

Supplementary Figure 1. Change in absolute number of T cells with exhaustion markers and Tregs post clofarabine (post clo) with respect to pre clofarabine(pre-Clo). A. Absolute numbers of $PD1^{+}CD4$ T cells. B. Absolute number of $LAG3^{+}CD4$ T cells. C. Absolute numbers of $TIM3^{+}CD4$ T cells. D. Absolute numbers of $FOXP3^{+}CD25^{+}CD4$ T cells (Tregs)

Supplementary Figure 2. Upregulation of T cell and NK cell cytotoxic genes after lenalidomide therapy.Volcano Plot with upregulated(Right upper and lower quadrants) and downregulated targets(Left upper and lower quadrants).P-value was shown in Y axis and relative normalized expression was shown in X-axis. After lenalidomide therapy, significant up-regulations of genes were observed in IL-15, IFNG, CSF1, IL7R, and IL-2.

Supplementary Table 1: List of Fluorescent antibodies used in multicolor flow cytometry.