Dynamic change in Epstein-Barr virus DNA predicts prognosis in early stage natural killer/T-cell lymphoma with pegaspargase-based treatment: long-term follow-up and biomarker analysis from the NHL-004 multicenter randomized study

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SUPPLEMENTARY INFORMATION

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Tables S1. The biomarker population.

Biomarkers	**ESA (*n*=125)**	**MESA (*n*=123)**	**Total (*n*=248)**
Interim EBV DNA	111	106	217
Post-treatment EBV DNA	99	92	191
C-MYC	75	74	149
CD56	125	123	248
DNA-sequencing	65	63	128
WGS/WES	43	42	85
Targeted sequencing	22	21	43
RNA-sequencing	44	43	87

Abbreviations: ESA, etoposide, dexamethasone, and pegaspargase; MESA, methotrexate, etoposide, dexamethasone, and pegaspargase; EBV, Epstein-Barr Virus; WGS, whole genome sequencing; WES, whole exome sequencing.

Table S2. The interim EBV DNA population and comparison with entire cohort.

Characteristics	Biomarker population (*n*=217)	The mITT population (*n*=248)	*p* value
Gender, No. (%)			0.918
Female	63 (29.0)	70 (28.2)	
Male	154 (71.0)	178 (71.8)	
Age, year, No. (%)			1.000
≤60	182 (83.9)	207 (83.5)	
>60	35 (16.1)	41 (16.5)	
B symptoms, No. (%)			1.000
Yes	84 (38.7)	95 (38.3)	
No	133 (61.3)	153 (61.7)	
ECOG, No. (%)			0.816
0-1	209 (96.3)	237 (95.6)	
≥ 2	8 (3.7)	11 (4.4)	
Ann Arbor stage, No. (%)			0.924
I	134 (61.8)	155 (62.5)	
II	83 (38.2)	93 (37.5)	
Local tumor invasion, No. (%)			0.925
Yes	88 (40.6)	99 (39.9)	
No	129 (59.4)	149 (60.1)	
Elevated lactic dehydrogenase, No. (%)			0.704
Yes	87 (40.1)	95 (38.3)	
No	130 (59.9)	153 (61.7)	
Ki67, No. (%)			1.000
≤ 50%	50 (23.0)	58 (23.4)	
> 50%	167 (77.0)	190 (76.6)	
International Prognostic Index, No. (%)			1.000
Low	199 (91.7)	227 (91.5)	
Intermediate-low	16 (7.4)	18 (7.3)	
Intermediate-high	2 (0.9)	3 (1.2)	
PINK-E, No. (%)			0.870
Low	199 (91.7)	226 (91.1)	
Intermediate	18 (8.3)	22 (8.9)	
Nomogram-revised risk index, No. (%)			0.990
Low	40 (18.4)	48 (19.4)	
Intermediate-low	84 (38.7)	97 (39.1)	
Intermediate-high	67 (30.9)	74 (29.8)	
High	26 (12.0)	29 (11.7)	
CA, No. (%)			0.951
I	73 (33.6)	87 (35.1)	
II	61 (28.1)	68 (27.4)	
III	83 (38.3)	93 (37.5)	
Final response			0.744
CR/PR	199 (91.7)	217 (87.5)	
SD/PD	18 (8.3)	23 (9.3)	
5y-progression-free survival rate	80.5%	77.6%	
5y-overall survival rate	86.4%	83.1%	

Abbreviations: EBV, Epstein-Barr Virus; mITT, modified intention-to-treat; ECOG, Eastern Cooperative Oncology Group; PINK-E, Prognostic index of natural-killer lymphoma-Epstein-Barr Virus; CA, Chinese Southwest Oncology Group and Asia Lymphoma Study Group ENKTL system; CR, complete remission; PR, partial remission; SD, stable disease; PD, progressive disease; *p* value were compared using Fisher's exact test.

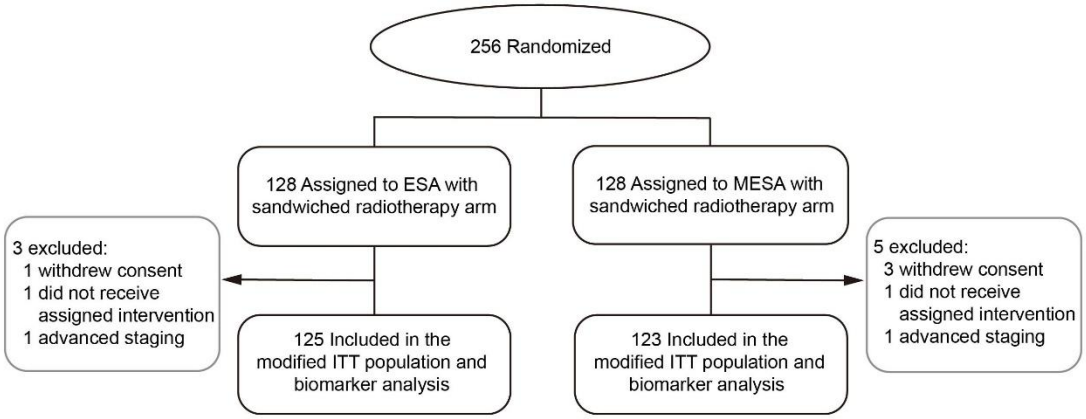

Figure S1. CONSORT Diagram.

ESA, etoposide, dexamethasone, and pegaspargase; MESA, methotrexate, etoposide, dexamethasone, and pegaspargase; mITT, modified intention-to-treatment.

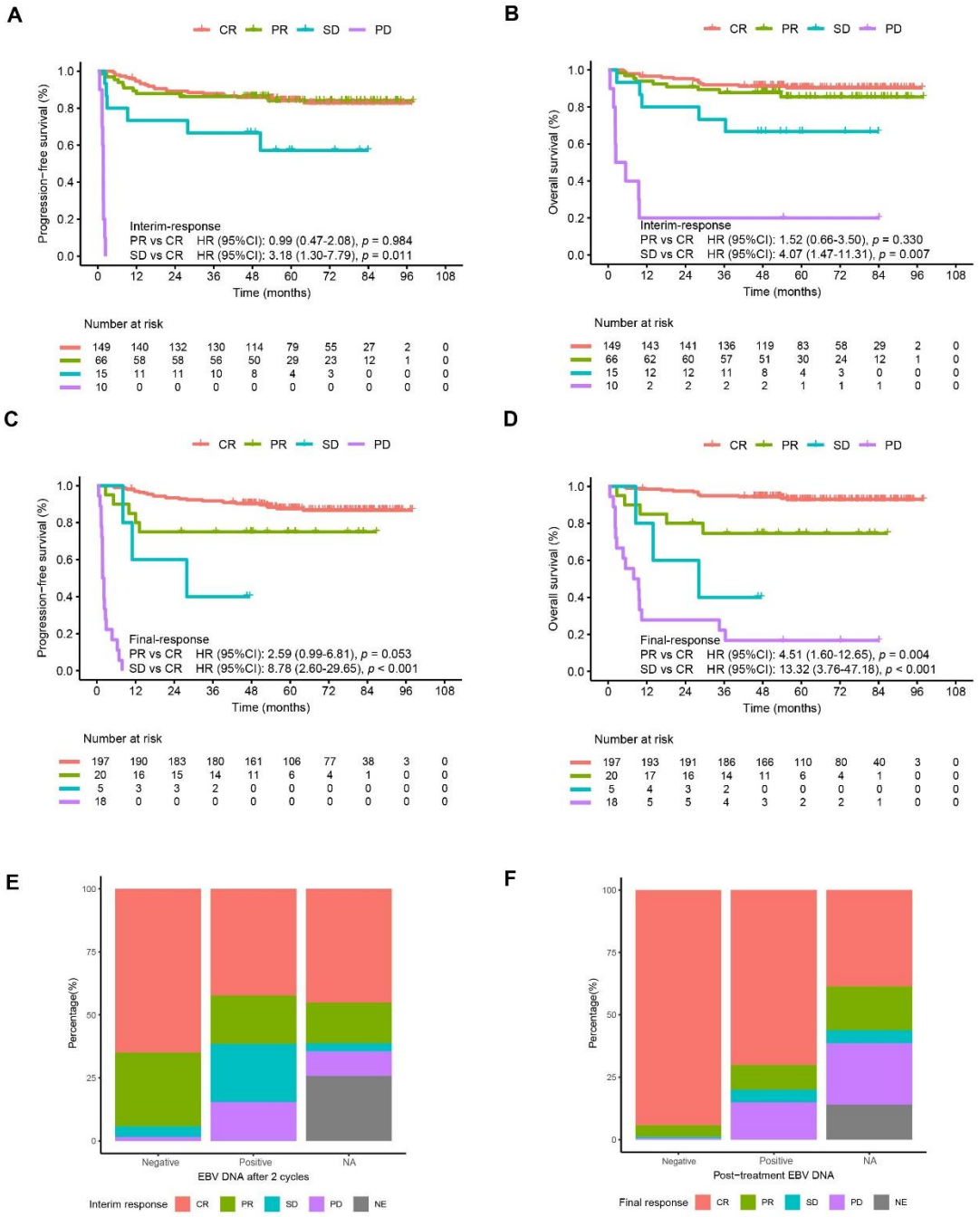

Figure S2. Progression-free survival and overall survival of Response Subgroups.

Kaplan-Meier survival curve of progression-free survival (PFS) (**A**) and overall survival (OS) (**B**) according to interim response. Kaplan-Meier survival curve of PFS (**C**) and OS (**D**) according to final response. (**E**) Correlation of interim EBV DNA and interim response. (**F**) Correlation of post-treatment EBV DNA and final response. HR, hazard ratio; CI, confidence interval; EBV, Epstein-Barr Virus; CR, complete remission; PR, partial remission; SD, stable disease; PD, progressive disease; NA, not applicable; NE, not evaluated.

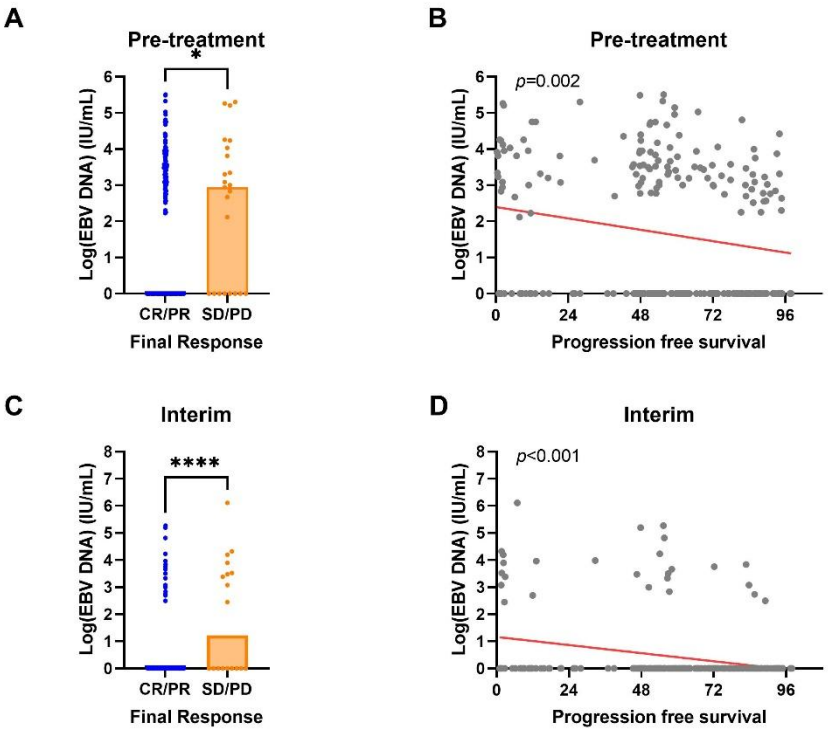

Figure S3. The EBV DNA load and outcomes

(**A**) Association of pre-treatment EBV DNA load with final response. (**B**) Correlation of pre-treatment EBV DNA load with progression-free survival. (**C**) Association of interim EBV DNA load with final response. (**D**) Correlation of interim EBV DNA load with progression-free survival. EBV, Epstein-Barr Virus.

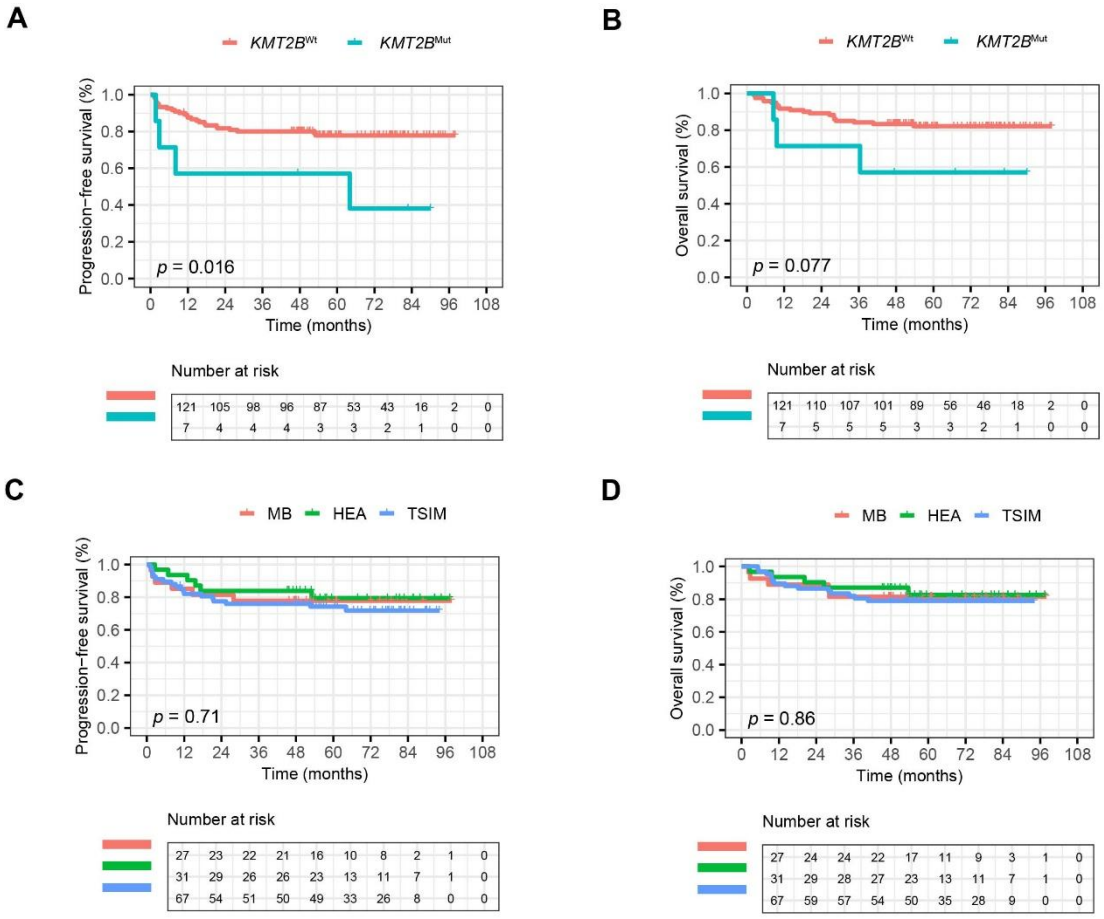

Figure S4. Genetic mutations and clinical outcomes.

Kaplan-Meier survival curve of progression-free survival (PFS) (**A**) and overall survival (OS) (**B**) according to *KMT2B* mutation. Kaplan-Meier survival curve of PFS (**C**) and OS (**D**) according to molecular subtypes.

Table S3. The baseline characteristics of patients according to interim EBV DNA.

	Interim EBV DNA		
Characteristics	**Negative (*n* = 191)**	**Positive (*n* = 26)**	***p* value**
Gender, No. (%)			0.020
Female	50 (26.2)	13 (50.0)	
Male	141 (73.8)	13 (50.0)	
Age, year, No. (%)			0.776
≤60	159 (83.2)	23 (88.5)	
>60	32 (16.8)	3 (11.5)	
B symptoms, No. (%)			0.675
Yes	73 (38.2)	11 (42.3)	
No	118 (61.8)	15 (57.7)	
ECOG, No. (%)			0.246
0-1	185 (96.9)	24 (92.3)	
≥ 2	6 (3.1)	2 (7.7)	
Ann Arbor stage, No. (%)			1.000
I	118 (61.8)	16 (61.5)	
II	73 (38.2)	10 (38.5)	
Local tumor invasion, No. (%)			1.000
Yes	78 (40.8)	10 (38.5)	
No	113 (59.2)	16 (61.5)	
Elevated lactic dehydrogenase, No. (%)			0.395
Yes	79 (41.4)	8 (30.8)	
No	112 (58.6)	18 (69.2)	
Ki67, No. (%)			0.142
≤ 50%	41 (21.5)	9 (34.6)	
> 50%	150 (78.5)	17 (65.4)	
International Prognostic Index, No. (%)			0.085
Low	174 (91.1)	25 (96.2)	
Intermediate-low	16 (8.4)	0 (0)	
Intermediate-high	1 (0.5)	1 (3.8)	
PINK-E, No. (%)			0.459
Low	176 (92.1)	23 (88.5)	
Intermediate	15 (7.9)	3 (11.5)	
Nomogram-revised risk index, No. (%)			0.555
Low	33 (17.3)	7 (26.9)	
Intermediate-low	76 (39.8)	8 (30.8)	
Intermediate-high	58 (30.4)	9 (34.6)	
High	24 (12.5)	2 (7.7)	
CA, No. (%)			1.000
I	64 (33.5)	9 (34.6)	
II	54 (28.3)	7 (26.9)	
III	73 (38.2)	10 (38.5)	
Type, No. (%)			0.200
MB	26 (23.6)	1 (6.7)	
HEA	25 (22.7)	6 (40.0)	
TSIM	59 (53.7)	8 (53.3)	
Arm, No. (%)			0.211
ESA	101 (52.9)	10 (38.5)	
MESA	90 (47.1)	16 (61.5)	
Final response, No. (%)			<0.001
CR/PR	182 (95.3)	17 (65.4)	
SD/PD	9 (4.7)	9 (34.6)	

Abbreviations: EBV, Epstein-Barr Virus; ECOG, Eastern Cooperative Oncology Group; PINK-E, Prognostic index of natural-killer lymphoma-Epstein-Barr Virus; CA, Chinese Southwest Oncology Group and Asia Lymphoma Study Group ENKTL system; ESA, etoposide, dexamethasone, and pegaspargase; MESA, methotrexate, etoposide, dexamethasone, and pegaspargase; CR, complete remission; PR, partial remission; SD, stable disease; PD, progressive disease; *p* value were compared using Fisher's exact test.

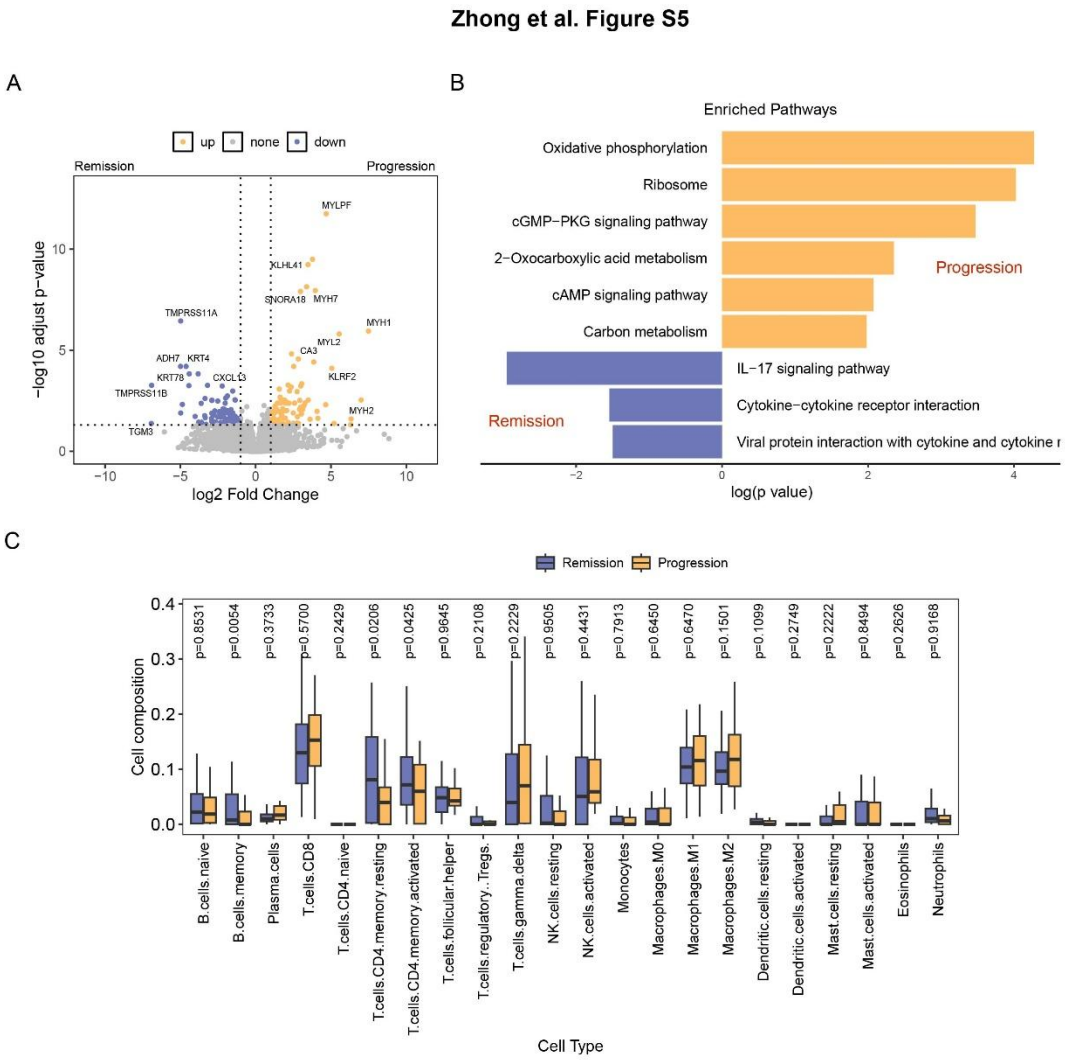

Figure S5. Transcriptomic signatures associated with progression.

(**A**) Differentially expressed genes between progression and remission. (**B**) Gene Set Enrichment Analysis revealed differentially pathway alterations between progression and remission. (**C**) Infiltration of immune cells between progression and remission.

Table S4. The baseline characteristics of patients according to progression-free survival time.

	Progression-free survival time		
Characteristics	**Early (≤12m) (*n* = 35)**	**Late (>12m) (*n* = 20)**	***p* value**
Gender, No. (%)			0.728
Female	8 (22.9)	3 (15.0)	
Male	27 (77.1)	17 (85.0)	
Age, years, No. (%)			1.000
≤ 60	30 (85.7)	18 (90.0)	
> 60	5 (14.3)	2 (10.0)	
B symptoms, No. (%)			1.000
Yes	15 (42.9)	8 (40.0)	
No	20 (57.1)	12 (60.0)	
ECOG, No. (%)			0.529
0-1	33 (94.3)	20 (100)	
≥ 2	2 (5.7)	0 (0)	
Ann Arbor stage, No. (%)			0.270
I	23 (65.7)	10 (50.0)	
II	12 (34.3)	10 (50.0)	
Local tumor invasion, No. (%)			0.273
Yes	18 (51.4)	7 (35.0)	
No	17 (48.6)	13 (65.0)	
Elevated lactic dehydrogenase, No. (%)			0.391
Yes	13 (37.1)	5 (25.0)	
No	22 (62.9)	15 (75.0)	
Pre-treatment EBV DNA, No. (%)			0.575
Positive	21 (60.0)	10 (50.0)	
Negative	14 (40.0)	10 (50.0)	
Ki67, No. (%)			1.000
≤ 50%	8 (22.9)	4 (20.0)	
> 50%	27 (77.1)	16 (80.0)	
International Prognostic Index, No. (%)			0.292
Low	31 (88.6)	20 (100)	
Intermediate-low	3 (8.6)	0 (0)	
Intermediate-high	1 (2.8)	0 (0)	
PINK-E, No. (%)			0.399
Low	30 (85.7)	19 (95.0)	
Intermediate	5 (14.3)	1 (5.0)	
Nomogram-revised risk index, No. (%)			0.282
Low	6 (17.2)	2 (10.0)	
Intermediate-low	14 (40.0)	12 (60.0)	
Intermediate-high	11 (31.4)	6 (30.0)	
High	4 (11.4)	0 (0)	
CA, No. (%)			0.511
I	10 (28.6)	4 (20.0)	
II	13 (37.1)	6 (30.0)	
III	12 (34.3)	10 (50.0)	
Type, No. (%)			0.329
MB	4 (22.2)	2 (16.7)	
HEA	2 (11.1)	4 (33.3)	
TSIM	12 (66.7)	6 (50.0)	
Arm, No. (%)			0.159
ESA	13 (37.1)	12 (60.0)	
MESA	22 (62.9)	8 (40.0)	

Abbreviations: ECOG, Eastern Cooperative Oncology Group; EBV, Epstein-Barr Virus; PINK-E, Prognostic index of natural-killer lymphoma-Epstein-Barr Virus; CA, Chinese Southwest Oncology Group and Asia Lymphoma Study Group ENKTL system; ESA, etoposide, dexamethasone, and pegaspargase; MESA, methotrexate, etoposide, dexamethasone, and pegaspargase; *p* value were compared using Fisher's exact test.

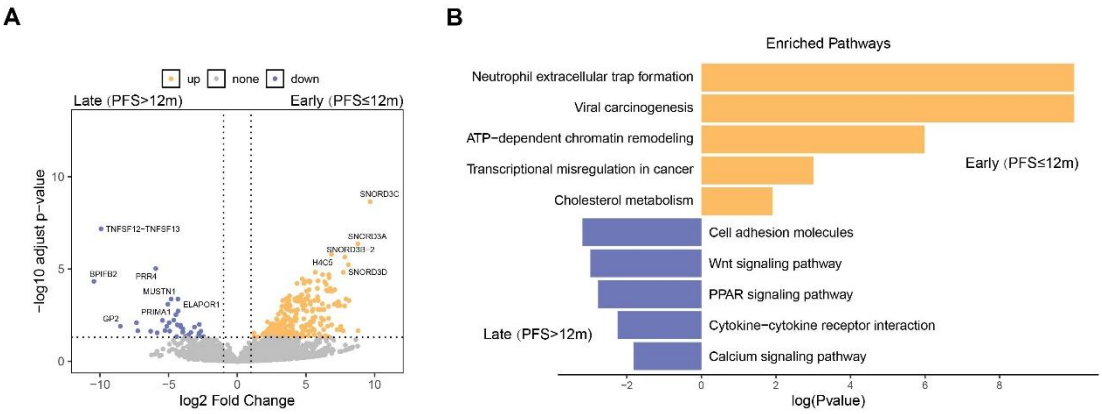

Figure S6. Transcriptomic signatures associated with early progression.

(**A**) Differentially expressed genes between early (progression-free survival [PFS] <= 12months) and late (PFS > 12months) progression. (**B**) Gene Set Enrichment Analysis revealed differentially pathway alterations between early (PFS <= 12months) and late (PFS > 12months) progression.

Table S5. Long-term survival of regimens in newly diagnosed early-stage NKTCL.

	RT-DeVIC (J Clin Oncol 2016)	VIDL (Ann Hematol 2014)	GELOX (Cancer 2013/ Oncol Lett 2015)	DDGP (JAMA Oncol 2022)	MESA (This study)	ESA (This study)
No. of patients	150	24	27	40	123	125
Median Age	56 (16-83)	47 (22-71)	Not reported	41 (Not reported)	46 (15-70)	50 (14-70)
Age > 60 years	55 (37%)	6 (20%)	4 (14.8%)	23 (57.5%)	15 (12.2%)	26 (20.8%)
Male	111 (74%)	20 (67%)	16 (59.3%)	23 (%)	94 (76.4%)	84 (67.2%)
Ann Arbor stage II	Not reported	9 (30%)	9 (33.3%)	Cancer stage III:25 (62.5%)	45 (36.6%)	48 (38.4%)
B symptoms	51 (35%)	9 (30%)	10 (37%)	19 (47.5%)	43 (35.0%)	52 (41.6%)
ECOG PS > 1	8 (5%)	1 (3%)	4 (14.8%)	10 (25%)	5 (4.1%)	6 (4.8%)
Elevated LDH	42 (28%)	8 (27%)	3 (11.1%)	17 (42.5%)	48 (39.0%)	47 (37.6%)
Positive pre-treatment EBV DNA	Not reported	Not reported	Not reported	18 (45.0%)	58 (47.2%)	57 (45.6%)
ORR	81% (J Clin Oncol 2009)	90%	96.3%	90.0%	86.2%	88.8%
Median follow-up time	5.8 years	44 months	63.15 months	41.5 months	64 months	64 months
5y-PFS	61%	60%	74.0%	Not reported	74.9%	80.3%
5y-OS	72%	73%	85.0%	74.3%	80.9%	85.1%

Abbreviations: DeVIC, dexamethasone, etoposide, ifosfamide and carboplatin; VIDL, etoposide, ifosfamide, dexamethasone, L-asparaginase and cisplatin; GELOX, gemcitabine, L-asparaginase and oxaliplatin; DDGP, dexamethasone, cisplatin, gemcitabine, and pegaspargase; ESA, etoposide, dexamethasone, and pegaspargase; MESA, methotrexate, etoposide, dexamethasone, and pegaspargase;