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Clonal evolution and the risk of secondary myeloid neoplasia following chimeric antigen receptor T-cell therapy

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CR, FS, RG designed the project, RB, TM, SP designed and performed the laboratory work. AK performed statistical analysis, AR, KC, FS, MOR compiled the clinical data. FS, AR, KC and CR wrote the manuscript. All authors edited and reviewed the manuscript.

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TO THE EDITOR:

Chimeric antigen receptor T-cell therapy (CAR-T) has revolutionized management of relapsed/refractory non-hodgkin lymphoma (NHL)[1,] Whilst immune-effector-cell-associated hematological toxicity (ICAHT) is the commonest cause of cytopenias post-CAR-T, secondary treatment-associated myeloid neoplasms (tMN) have been reported with increasing frequency [2- 5.]

tMN is a well-recognized complication of DNA-damaging chemotherapy, classically arising 5-7 years post-exposure, and prognosis is extremely poor (<1 year overall survival(OS))[6,7]. In NHL patients treated with autologous stem cell transplant (ASCT), 10-year tMN incidence can be as high as 12-24%, with older/heavily pre-treated patients at highest risk [8-9]. Whilst pivotal CAR-T studies did not initially report tMN as an adverse event, emerging real-world data suggests an incidence of 3-6%[2,4,]. In contrast to the post-ASCT experience, CAR-T patients appear to have short latency between CAR-T infusion and tMN onset e.g. 3-10 months [4] , compared to 4-5 years in the post-ASCT setting, and an extremely poor prognosis of only 3-8 months following tMN diagnosis [4], compared to 13-15 months in the post-ASCT setting. Clonal haematopoeisis of indeterminate potential (CHIP) has been observed in some pre-CAR-T patients and may contribute to tMN risk[9]; additionally mutations in DNA repair mechanisms have been identified in cases of post CAR-T tMN [2, 10] . Clonal evolution has been identified in several cases of post-CAR-T tMN [2-3,9].

Identification of risk factors for development of post-CAR-T tMN is of paramount importance to flag high-risk subgroups who may benefit from closer monitoring post-CAR-T, and to aid pre-CAR-T patient counselling.

Here we describe our single-centre experience of post-CAR-T tMN in 10 NHL patients. Using an age- and sex-matched control group, we identify potential risk factors for tMN, and begin to characterize underlying driver mutations and clonal evolution in this cohort.

Demographics/disease characteristics/baseline CAR-HAEMATOTOX (HT) scores, were assessed for 10 cases of post-CAR-T myeloid malignancies (tMN) (WHO/5th-edition) arising between 2019-2024 at UCLH and for a 3:1 age-/sex-matched control cohort who received CAR-T at UCLH but did not develop tMN (Supplementary material, figure S1). RNASeq (Archer Pan-Heme FusionPlex NGS) was conducted on pre-CAR-T/post-CAR-T samples (blood/bone marrow(BM)/lymph node(LN). Ethical approval for the study data was granted by Derby Research Ethics Committee(REC) reference 24/EM/0221, IRAS project ID 336254. To assess baseline and post-CAR-T risk factors separately, time to development of tMN was measured as the interval between CAR-T infusion or day 28 respectively and date of tMN diagnosis/death/date last seen alive using competing risks survival analysis (Fine&Grey) with death (without tMN) included as a competing risk. Associations between

number of prior therapeutic lines/HT/ASCT were assessed using linear regression and Wilcoxon Mann-Whitney testing. All analyses were performed in STATA version 18.0 (STATACORP, Texas), with two-sided $p<0.5$ indicating statistical significance.

Among 403 CAR-T infused NHL patients at UCLH, 10 cases of post-CAR-T myeloid malignanices conditions (tMN) were observed (incidence, 2.48%). All patients within this cohort were treated with CD19-directed CAR-T, 9/10 (90%) of tMN were treated with commerical CAR-T products with CD28 co-stimulatory domains, 1/10 (10%) patients were treated with AUTO-1 containing a 4-1BB co-stimulatory domain (Table 1, table S1). All patients received standard intensity lymphodepletion with Fludarabine and Cyclophosphamide prior to CAR-T (Supplementary material, table S1). Median age was 54 years (range,33-67), 6/10 were male, disease stage was III-IV in 8/10, and median baseline LDH was 244 U/L (range,155-462) (Table 1). 2/10 patients who developed tMN relapsed with NHL and received further therapy at 14 and 16 months post CAR-T (Supplementary material, table S1). tMN was diagnosed at a median of 11.2 months(IQR:5.2–21.2) post-CAR-T and included acute myeloid leukaemia(AML) in 2/10, and myelodysplastic syndrome(MDS) with bi-allelic TP53 inactivation, low blasts or hypoplasia in 1/10, 6/10, and 1/10 cases respectively. Median OS post-tMN diagnosis was 8.1 months [IQR:6.4-24.3], 70% of the tMN cohort are now deceased (Figure 1) and all deaths were due to tMN, with two patients having infectious complications (appendicitis, mucormycosis) listed as additional causes of death (table S1).

In 7/10 cases, pre-CAR-T biopsy material was available to test for baseline CHIP by NGS (1/7 pleural biopsy; 3/7 BM; 3/7 LN), and pre-CAR-T driver mutations of variant allele frequency (VAF) >2%[11] were identified in 4/7 patients (1/4 pleural biopsy;3/4 BM). These clones included PPM1D(2/4), TP53(1/4), TET2(1/4), ASXL1(1/4), RUNX1(1/4), and CEBPA(1/4), which persisted/increased at tMN diagnosis. In 3/10 patients with detectable non-driver CHIP at baseline, mutations arising at tMN diagnosis were TP53(1/3), DNTM3A(3/3), ZRSR(1/3), PPM1D(2/3) and ASXL1(1/3); this is illustrated in Figure 1. In 3/10 patients without baseline samples/detectable CHIP, mutations in ASXL1(2/3), DNMT3A(2/3), RUNX1(1/3) and BCOR(1/3) were identified by BM analysis at tMN diagnosis. We included non bone-marrow tissues such as pleural fluid and lymph node to allow us to demonstrate mutational yield across sample types. Individual patient characteristerics including tMN classification, IPSS risk group and tMN treatment are outlined in Table S1.

10/10 (100%) of tMN patients had complete response (CR) or partial response (PR) to CAR-T at D100 versus 26/30 (86.6%) in the control group (p 0.22). Univariable analysis comparing tMN patients to the control cohort (median follow-up,33.8 months [IQR:25.5–40.4],range:7-50.3) identified a significant association between pre-CAR-T factors such as prior ASCT, high baseline HT score (table 2, Figure S2), and number of prior therapeutic lines with risk of post-CAR-T tMN (Table 1&2). Small

patient numbers preclude full multivariable analysis, but pairwise comparisons identify baseline HT score (i.e. increase HT by 1 point: HR=1.47(1.02–2.14),p=0.041), and number of prior therapeutic lines (i.e. increase prior lines by 1 additional line: HR=2.01(1.24–3.25),p=0.005) as independent risk factors. In contrast, much of the excess risk from prior ASCT disappeared when adjusted for prior therapeutic lines (HR=2.31 (0.36–14.89),p=0.38). Higher peak lymphocyte count but not LDH was significantly associated with tMN (p=0.012), with a trend for higher CRS grade (p=0.053). There was no association with peak serum ferritin/CRP/ICANS grade, nor time to neutrophil recovery during month 1.

Published data suggests that tMN affects 3-6% of post-CAR-T patients, occurs with short latency, and clinical outcomes are extremely poor[10]. Proposed risk factors include advanced age, pre-treatment cytopenia(s)[2], multiple prior treatment lines[2,10], and the presence of pre-treatment CHIP 'driver mutations' in a subset of patients[2-3 ,9]. Here, using CAR-T for NHL in the 3rd-line setting, we confirm these observations in 10 cases of tMN, demonstrating an overall incidence of 2.48%, short latency (median 7.3months), and poor median OS (8.1 months).

Pre-treatment risk prediction vs age-matched controls shows that high baseline HT score and number of prior therapeutic lines (including ASCT) are associated with higher tMN risk. Future data on tMN incidence following 2nd-line (and even 1st-line) CAR-T for NHL will help determine whether CAR-T drives tMN risk independently of prior treatments. If the tMN signal is lower in 1st/2nd-line CAR-T, this may further support the use of CAR-T in earlier lines.

In our analysis, peak lymphocyte count (as surrogate for CAR-T expansion) was significantly associated with increased risk of tMN, whilst higher CRS grade showed a trend towards increased risk of tMN. Higher HT score at baseline was associated with tMN risk but peak post-infusion ferritin, which is often used as a marker of CAR-inflammation, was not. Whilst there is a paucity of mechanistic data to explain a pathophysiological link between CAR-T and tMN, it is hypothesized that CAR-T-associated inflammation may play a role, suppressing normal haematopoiesis[12] and provoking persistent cytopenias[13]. RNAseq identifies clonally expanded IFNγ-secreting CX3CR1+CD8+ T-cells which can suppress normal haemapoeisis[14]. Further, common myeloid CHIP/'first-hit' mutations in the epigenetic regulators ASXL1/TET2/DNMT3a/JAK2 can reprogramme myeloid cells to adopt a 'pro-inflammatory state'. Here, we identified pre-CAR-T driver mutations in 40% of the tMN cohort, of which 75% of had driver mutations in DNA-repair genes; this is reportedly more common in tMN vs de novo MN due to the selective advantage conferred by mutated DNA-repair genes on malignant cells when exposed to cytotoxic chemotherapy[15]. Our cohort was heavily pre-treated, which may synergise with the highly pro-inflammatory environment provoked by CAR-T and cause a rapid progression to tMN. Through further propagation of a pro-

inflammatory BM niche, CAR-T may confer CHIP clones with a proliferative advantage, favouring evolution of pre-existing CHIP to tMN, and potentially explaining how undetected CHIP clones pre-CAR T may become clinically apparent post-treatment. Consistent with the potential risk posed by inflammation, our analysis suggests that higher peak lymphocyte expansion post-CAR-T and possibly higher CRS grade are associated with higher tMN risk, and in the 4 patients with detectable pre-CAR-T tMN driver mutations, we observed clonal evolution following CAR-T infusion.

Study limitations include small numbers which preclude multivariable analysis. Due to the matched case-control design we were unable to assess the association of tMN with age or sex. We were unable to estimate the cumulative incidence of tMN and 2.48% may be an underestimate, particularly in recently infused patients. It will be essential to explore our findings further in large multi-centre studies and compare incidence of tMN with other treatment approaches such as an ASCT. Mutational data from non-bone marrow tissues could represent lymphoid rather than myeloid CHIP hence confounding interpretation, however this was included to describe longitudinal evolution of mutations and compare mutational yields across sample types.

Our data is of key importance in highlighting patient characteristics associated with post CAR-T tMN, which may guide decisions on CAR-T timing and inform patient counselling about risk. NGS screening pre-CAR-T is not standard practice but this may be prudent in heavily pretreated patients and those with high baseline HT scores. Those patients identified with CHIP and additional risk factors may benefit from post-treatment surveillance for tMN to allow earlier diagnosis and consideration of allogeneic stem cell transplantation in eligible candidates.

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TABLES:

Patient demographics and risk factors associated with treatment-associated myeloid neoplasm (tMN) development post Chimeric antigen receptor T cell (CAR-T).

Table 1: Baseline demographics of post CAR-T MN group including CAR-Hematotox (HT) scores and biochemical parameters post CAR-T infusion compared to an age-matched control cohort.

		Myeloid malignancies N=10	Control N=30
CAR-T product, N patients (%)			
	Axi-cel	8 (80.0)	28 (93.3)
	AUTO1	1 (10.0)	0
	Brexu-cel	1 (10.0)	0
	Tisa-cel	0	2 (6.7)
Age (years), median (IQR) range		54.0(50.0- 60.0) 33 - 67	55.0(49.0- 59.0) 33 - 62
Sex, N (%)			
	Male	6 (60.0)	18 (60.0)
	Female	4 (40.0)	12 (40.0)
Diagnosis, N (%)			
	De novo DLBCL	1 (10.0)	20 (69.0)
	tFL	4 (40.0)	7 (24.1)
	FL	2 (20.0)	1 (3.4)
	MCL	1 (10.0)	0
	tWM	1 (10.0)	0
	tLPL	1 (10.0)	0
	PMBCL	0	1 (3.4)
	Missing/unknown	0	1
Stage			
	0-II	2 (20.0)	8 (26.7)
	III-IV	8 (80.0)	22 (73.3)
LDH, N (%), units U/L			
	<ULN	3 (30.0)	11 (36.7)
	≥ULN<2xULN	6 (60.0)	15 (50.0)
	≥2xULN	1 (10.0)	4 (13.3)
	Median (IQR)	244.0(185.0-312.0)	247.0(203.0-286.0)
	range	*155 - 462*	*163 - 1168*
CAR-HEMATOTOX, median (IQR) range		3.5(2.0- 5.0) 0 - 6	1.0(0.0- 3.0) 0 - 5
Prior lines of therapy, N (range)		5(4-5)	2(2-3)

	3 - 6	*2 - 6*
Previous SCT, N (%)		
No	2 (20.0)	23 (76.7)
Autograft	8 (80.0)	6 (20.0)
Autograft and Allo	0	1 (3.3)
CRP baseline mg/L, median (IQR)	9.2(6.3- 13.0)	2.9(2.1- 13.7)
range	*4.10 - 47*	*0.5 - 70.7*
CRP peak, median (IQR)	37.9(29.4- 87.5)	62.2(25.9-161.2)
range	7.90 - 136.80	1.7 - 284.1
Change in CRP (%), median (IQR)	572.9(90.2-717.1)	1233.5(308.9-5851.3) *120.20 -*
range	*80.64 - 2171.43*	*17666.67*
Baseline Ferritin (ug/L), median (IQR)	678.5(135.0-1155.0)	532.0(179.0-1190.0)
range	*30 - 10487*	*40 - 1662*
Peak Ferritin (ug/L), median (IQR)	1298.0(1051.0-2312.0)	1025.0(574.0-2080.0)
range	*154 - 12468*	*90 - 3498*
Change in Ferritin (%), median (IQR)	261.8(182.5-545.4)	232.9(139.2-330.7)
range	*118.89 - 853.41*	*41.34 - 628.75*
Lymphocyte count at baseline (x 10^9/L) , median (IQR)	0.6(0.4- 0.8)	0.5(0.4- 0.6)
range	*0.18 - 1.67*	*0.15 - 1.42*
Lymphocyte count post CAR-T (absolute), median (IQR)	0.4(0.2- 0.5)	0.2(0.1- 0.3)
range	*0.070 - 1.07*	*0.020 - 0.79*
Change in Lymphocyte count (%), median (IQR)	38.9(29.8- 91.5)	39.0(24.6- 54.9)
range	*17.39 - 254.76*	*5.56 - 453.33*
Max CRS Grade, median (IQR)	2.0(1.0- 2.0)	1.0(1.0- 2.0)
range	*1 - 2*	*0 - 2*
Max ICANS Grade, median (IQR)	0.0(0.0- 0.0)	0.0(0.0- 0.0)
range	*0 - 3*	*0 - 3*

Key: HT score: CAR-Hematotox score, Axi-cel: Axicabtagene Ciloleucel, AUTO1: academic CAR-T product (CD19CAT-41BBζ), Brexu-cel: Brexucabtagene autoleucel, Tisa-cel: Tisagenlecleucel, t: transformed, DLBCL: Diffuse Large B Cell lymphoma, FL: Follicular Lymphoma, MCL: Mantle cell lymphoma, WM: Waldenström's Macroglobulinaemia, LPL: Lymphoplasmacytic Lymphoma, PMBCL: Primary mediastinal B cell lymphoma (PBMCL), LDH: lactate dehydrogenase, ULN: upper limit of normal, IQR: interquartile range, CRS: cytokine release syndrome, ICANS: Immune effector cell-associated neurotoxicity syndrome, Autograft: Autologous stem cell transplant, Allo: Allogeneic stem cell transplant

Table 2: Univariable analysis of risk factors for post CAR-T MN expressed as Events/ number(N) and hazard ratio (HR) (95% CI), p-value, separated into pre CAR-T and post CAR-T risk factors.

Risk factor	Events/N	HR (95% CI)	p-value
Pre-CAR-T			
Stage at CAR-T approval			
Stage 0-II	2/10	1.00	0.76
Stage III-IV	8/30	1.27 (0.27 – 5.99)	
Previous autograft			
No	2/25	1.00	0.010
Yes	8/15	7.63 (1.62 – 35.99)	
Prior lines of therapy	10/40	2.27 (1.48 – 3.47)	<0.001
CAR-HEMATOTOX score			
Low (0-1)	2/17	1.00	0.065
High (2+)	8/20	4.33 (0.91 – 20.55)	
CAR-HEMATOTOX score (continuous)	8/30	1.63 (1.11 – 2.41)	0.013
Biochemical parameters (pre-CAR-T)[1]			
LDH (U/L) (for an increase of 225 (ULN))	10/40	0.76 (0.27 – 2.19)	0.61
Ferritin (ug/L) (for an increase of 100)	8/35	1.02 (1.00 – 1.05)	0.055
CRP(mg/L) (for an increase of 5)	7/29	1.02 (0.84 – 1.23)	0.87
Lymphocytes x 10^9/L	10/40	6.45 (1.03 – 40.40)	0.047
Post CART[2]			
Biochemical parameters[1]			
Ferritin			
Peak (for an increase of 100)	10/35	1.02 (1.00 – 1.04)	0.098
Change[3] (for an increase of 50%)	8/30	1.14 (0.97- 1.35)	0.10
CRP			
Peak (for an increase of 5)	9/29	0.98 (0.93 – 1.03)	0.37
Change[3] (for an increase of 50%)	7/23	0.97 (0.94 – 1.02)	0.32
Lymphocytes			
Peak (for an increase of 5)	9/39	18.27 (1.89 – 176.35)	0.012
Change[3] (for an increase of 50%)	9/39	1.41 (0.97 – 2.05)	0.070
Toxicity			
CRS (max grade)			
0	0/3	-	0.053[4]
1	4/20	-	

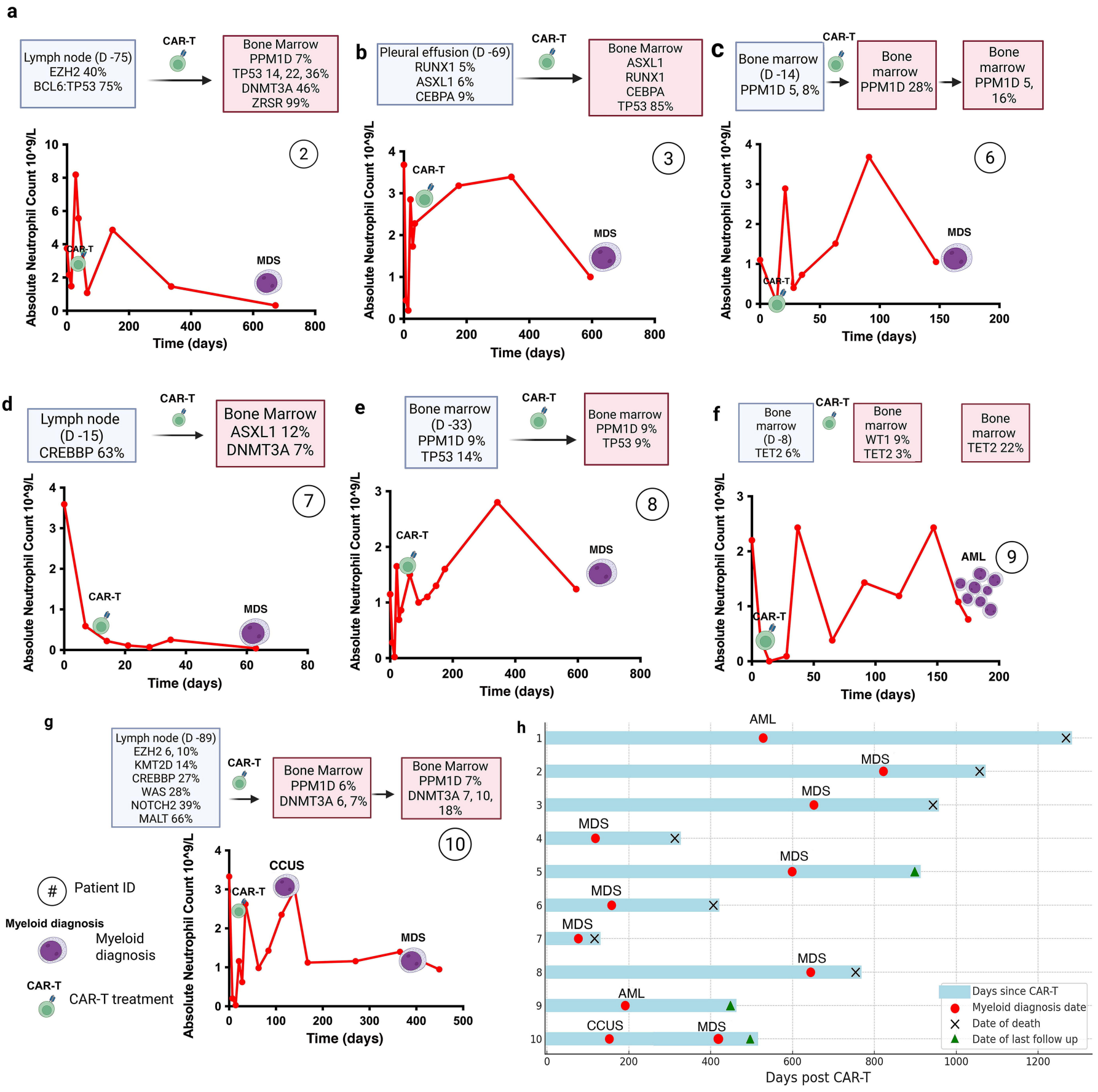
Risk factor	Events/N	HR (95% CI)	p-value
2	6/17	-	
ICANS (max grade)			
0	8/23	1.00	0.31[4]
1	1/5	0.76 (0.09 – 6.05)	

[1]HRs for continuous variables represent an increase in the number of units quoted e.g.for LDH this is the increase in risk for an increase of 225 i.e. the ULN for LDH. If no number is quoted, this is the HR for an increase of 1. For all percentage changes, an HR represents the increase in risk for a 50% increase.
[2]Time measured from day 28 to allow peak measurement for biochemical parameters and recording of CRS and ICANS.
[3]Adjusted for baseline value
[4]Log-rank test for trend

Key: CAR-HEMATOTOX (HT) score: derived from baseline absolute neutrophil count, platelet count, haemoglobin, C-reactive protein (CRP) (mg/L) and ferritin (ug/L). HR: hazard ratio, CI: confidence interval, LDH: lactate dehydrogenase (U/L), CRS: cytokine release syndrome, ICANS: Immune effector cell-associated neurotoxicity syndrome, Auto: autologous stem cell transplant, Allo: allogeneic stem cell transplant

FIGURE LEGENDS:

Figure 1: Clonal Evolution and patient characteristics. **(a-g):** clonal evolution over time plotted against absolute neutrophil count in 7/10 patients with pre-CAR-T and post chimeric antigen receptor T cell (CAR-T) treatment-associated myeloid neoplasm (tMN) biopsy material evaluated by next generation sequencing (NGS), non-bone marrow tissue is included to describe longitudinal mutational evolution, time on the x axis relates to number of days from the first mutational analysis.


a
Lymph node (D -75)
EZH2 40%
BCL6:TP53 75%
CAR-T
Bone Marrow
PPM1D 7%
TP53 14, 22, 36%
DNMT3A 46%
ZRSR 99%
b
Pleural effusion (D -69)
RUNX1 5%
ASXL1 6%
CEBPA 9%
CAR-T
Bone Marrow
ASXL1
RUNX1
CEBPA
TP53 85%
c
Bone marrow
(D -14)
PPM1D 5, 8%
CAR-T
Bone
marrow
PPM1D 28%
Bone
marrow
PPM1D 5,
16%
Absolute Neutrophil Count 10^9/L
Time (days)
CAR-T
MDS
2
3
6
d
Lymph node
(D -15)
CREBBP 63%
CAR-T
Bone Marrow
ASXL1 12%
DNMT3A 7%
e
Bone marrow
(D -33)
PPM1D 9%
TP53 14%
CAR-T
Bone marrow
PPM1D 9%
TP53 9%
f
Bone
marrow
(D -8)
TET2 6%
CAR-T
Bone
marrow
WT1 9%
TET2 3%
Bone
marrow
TET2 22%
AML
7
8
9
g
Lymph node (D -89)
EZH2 6, 10%
KMT2D 14%
CREBBP 27%
WAS 28%
NOTCH2 39%
MALT 66%
CAR-T
Bone Marrow
PPM1D 6%
DNMT3A 6, 7%
Bone Marrow
PPM1D 7%
DNMT3A 7, 10,
18%
10
CCUS
Patient ID
Myeloid diagnosis
Myeloid
diagnosis
CAR-T
CAR-T treatment
h
AML
MDS
CCUS
Days since CAR-T
Myeloid diagnosis date
Date of death
Date of last follow up
Days post CAR-T

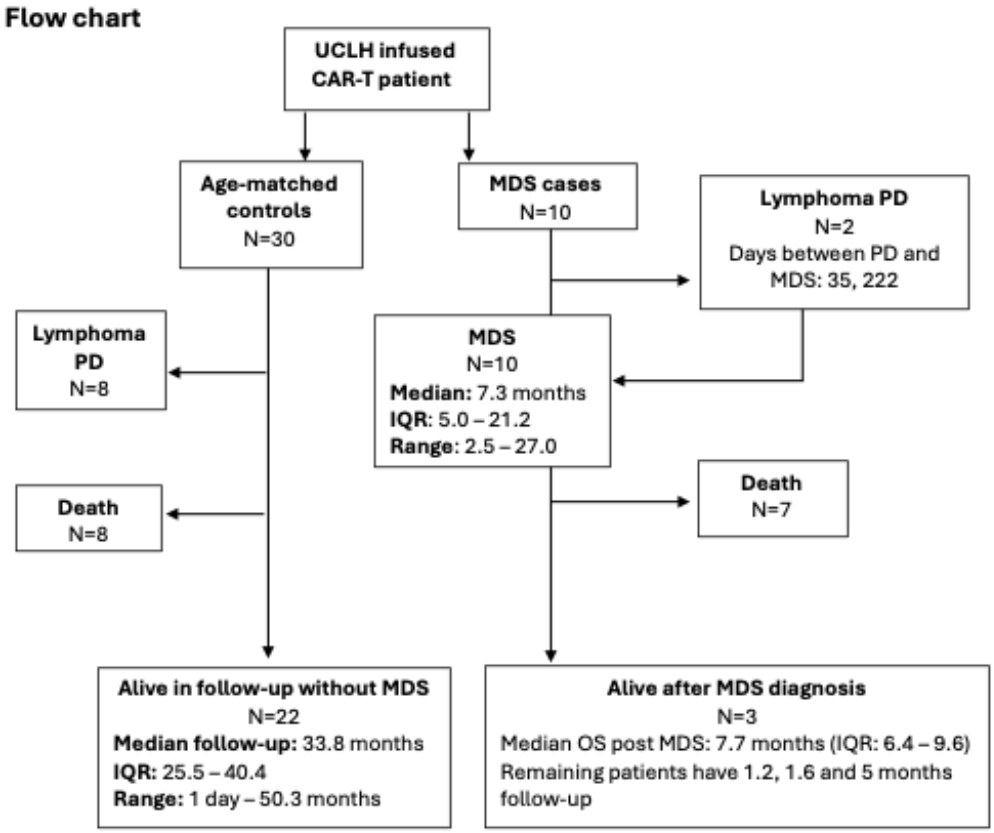


Figure S1: Consort diagram showing clinical outcomes for CAR-T patients diagnosed with tMN compared with an age-matched control cohort.

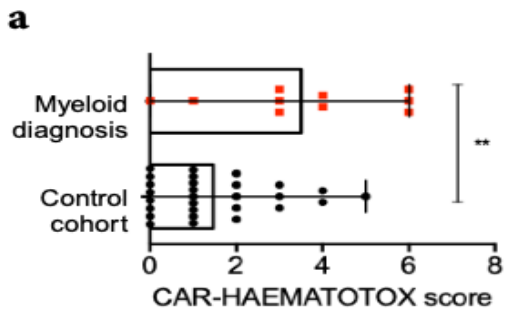


Figure S2 **(a)** HT score Myeloid diagnosis ‘MN’ cohort vs control cohort, statistics are mann-whitney test, two tailed p value 0.003, median with range

	Age at tMN diagnosis	Primary diagnosis	CAR-T product and conditioning used	Prior lines of therapy	Best response to CAR-T	Lymphoma Relapse post CAR-T	Peak Ferritin (ug/L)	Myeloid malignancy	Myeloid mutations (VAF%)	Treatment for MDS	Survival (months)	Cause of death
1	36	DLBCL (progression from Follicular lymphoma)	Axi-cel (Flu/Cy)	CVP, CHOP, Ofatumumab, ESHAP, IVE, LEAM Auto, Idelalisib, Bendamustine, Obinutuzamb, RT, TBI RIC haplo 2021	CR	Yes	2312	Therapy related AML	ASXL1 truncation (5%)	Ven-Aza	24	AML
2	62	DLBCL (progression from Follicular lymphoma)	Axi-cel (Flu/Cy)	Chlorambucil, Ritiuximab, R-CHOPx4, BEAM Auto, R-DHAP	CR	No	751	Myelodysplastic Syndrome IPSS 1.5	PPM1D (6.7%), TP53 (3 variants 22.1%, 35.8%, 13.9%), Pathogenic splice variant DNMT3A (46.1%), ZRSR2 (99.6%)	Azacytidine	8	MDS
3	49	Follicular Lymphoma	Axi-cel (Flu/Cy)	RCHOPx6, R-ICEx2, DHAPx2, BEAM Auto, R-GDP, R-Bendax2, Lenalidomide, Pola-BR	CR	No	1051	Therapy Related MDS IPSS 2 R-IPSS 5	TP53 mutation (C176S 85%), CNV pattern gain Chr 21	Azacytidine	12	MDS
4	67	Transformed LPL	Axi-cel (Flu/Cy)	Fludarabine x5, R-CPx8, R-CHOPx4, ESHAPx1, R-GDPxx3, BEAM Auto, RT	CR	No	1344	Myelodysplastic Syndrome IPSS 2 R-IPSS 5		Azacytidine	6	MDS

5	60	Transformed WM	Axi-cel (Flu/Cy)	Rituximab, R-CHOPx4, RT, R-ESHAPx2	CR	No	12468	Therapy related MDS IPSS 1	ASXL1mutation G646WfsTER12 (17%)	Planned for allo-SCT	9	N/A
6	61	DLBCL (progression from Follicular lymphoma)	Axi-cel (Flu/Cy)	Rituximab+Chlorambucil, 6xR-Benda, LEAM Auto, R-CHOPx6, R-ICE, RT	CR	No	1149	Secondary MDS IPSS 2 R-IPSS 4	Monosomy 7 (16%) Myeloid NGS PPM1D p.Ala481ProfsTer2 (28%)	Azacytidine	8	Mucormycosis MDS
7	51	Follicular Lymphoma	AUTO-1 (Flu/Cy)	RCHOPx6, R-GDP, Glofit+41BB ab (Trial P41072), R-Benda	CR	No	154	MDS IPSS 1 R-IPSS 3	DNMT3A (8%), LN: ASXL G646WfsTer12 variant LN NGS, FLIT3-ITD clone	Nil	1	Appendicitis MDS
8	58	Mantle Cell Lymphoma	Brexu-cel (Flu/Cy)	Nordic protocol, LEAM AUTO, Rituximab, Ibrutinib, Venetoclax	CR	Yes	1313	Therapy related MDS IPSS 1.5 R-IPSS 5	PPM1D p.Arg581Ter (9%), TP53 p.Arg273Leu (9%)	Nil	0	MDS
9	50	Diffuse Large B-cel Lymphoma	Axi-cel (Flu/Cy)	RCHOP, R-IVAC/RCODOX/M, R-GDP, LEAM ASCT, RBP	PR	No	1283	Therapy related AML	NUP98-NSD1 translocation; WT1 mutation; TET2 mutation	Ven-Aza And allo-SCT	8	N/A
10	52	Follicular	Axi-cel (Flu/Cy)	FL: Rbenda x6, Ritux maintenance, R-CHOP for relapse, LEAM Auto	CR	No	3554	Therapy related MDS	TET2 p.Ser714Ter (47%),	Nil	1	N/A

		Lymphoma		10/11/20; tFL: GDPx2, Epcoritimab x4				IPSS 1.5 R-IPSS 5.5	TET2 p.Arg544Ter (40%) and SRSF2 p.Pro95Leu DNMT3A p.Pro633Leu (18%), DNMT3A p.Asp658Tyr (10%), PPM1D p.Cys478Ter (7%) and DNMT3A c.2173_2173+1del splice donor and coding sequence (6%) variants.			

Table S1. Detailed patient characteristics for patients who developed myeloid malignancy post CAR-T cells therapy. WM: Waldenstrom's Macroglobulinaemia. LPL: Lymphoplasmacytic Lymphoma. Axi-cel: Axicabtagene Ciloleucel. AUTO-1: CD19CAT-41BBζ. Flu/Cy: Fludarabine/Cyclophosphamide. CVP: cyclophosphamide, vincristine, and prednisolone. R-: Rituximab. CHOP: cyclophosphamide, doxorubicin hydrochloride (hydroxydaunomycin), vincristine sulfate (Oncovin) and prednisolone. ESHAP: Etoposide, Solumedrol, High-dose Ara-C, and Platinum. IVE: Ifosfamide, Etoposide, and Epirubicin. LEAM-Auto: Lomustine, Etoposide, Ara-C (cytarabine), and Melphalan autologous stem cell transplantation. RT: Radiotherapy. TBI: Total Body Irradiation. Haplo: Haploidentical stem cell transplantation. Benda: Bendamustine. GDP: Gemcitabine, Dexamethasone, and Cisplatin. ICE: Ifosfamide, Carboplatin, and Etoposide. Pola-BR: Polatuzumab Vedotin, Bendamustine, and Rituximab. IVAC: Ifosfamide, Etoposide, and high-dose Cytarabine. CODOX/M: Cyclophosphamide, Vincristine (Oncovin), Doxorubicin, high-dose Methotrexate, and cytarabine (Ara-C).(R)IPSS: (Revised) International Prognostic Scoring System. AML: Acute Myeloid Leukaemia. MDS: Myelodysplastic Syndrome.