Supplemental Appendix:

1.0. Methods

We illustrate two cases from patients both treated on a Phase I study of CD22 CAR T-cells (NCT02315612), with the second patient also being involved in a Phase I study of CD19 CAR T-cells (NCT02028455) and a transplant trial incorporating use of blinatumomab on a haploidentical transplant platform (NCT02790515). All protocols were IRB approved by their respective institutions and consent and/or assent was obtained as per local guidelines.

1.1. CAR T-cell quantification

Flow cytometry was used to quantitate CD22 CAR T-cells in blood, bone marrow, and CSF using a CD22-Fc fusion protein (R&D Systems, Minneapolis, MN),[8] and circulating CAR T-cell numbers were calculated based upon absolute lymphocyte counts. Quantification of CAR T-cell number was also carried out using Droplet Digital PCR (ddPCR) with primer/probe specific for the CAR-T lentivector and normalized to total cell number measured by primer/probe for human reference gene *MKL2* as described in Shah et al.[7] Each CAR T-cell is assumed to carry a single integrated CAR-T lentivector. Each human cell carries 2 alleles of *MKL2* gene, and the total cell number in the input is calculated as half of the *MKL2* positive counts. Primers and probe were synthesized by Integrated DNA Technologies, Inc. (Coralville, IA). ddPCR assay was performed using BioRad Droplet Digital PCR system (Hercules, CA) Specific primers and probe are described in Supplemental Methods.

1.2. List of primers and probe used for CAR T-cell detection and quantification

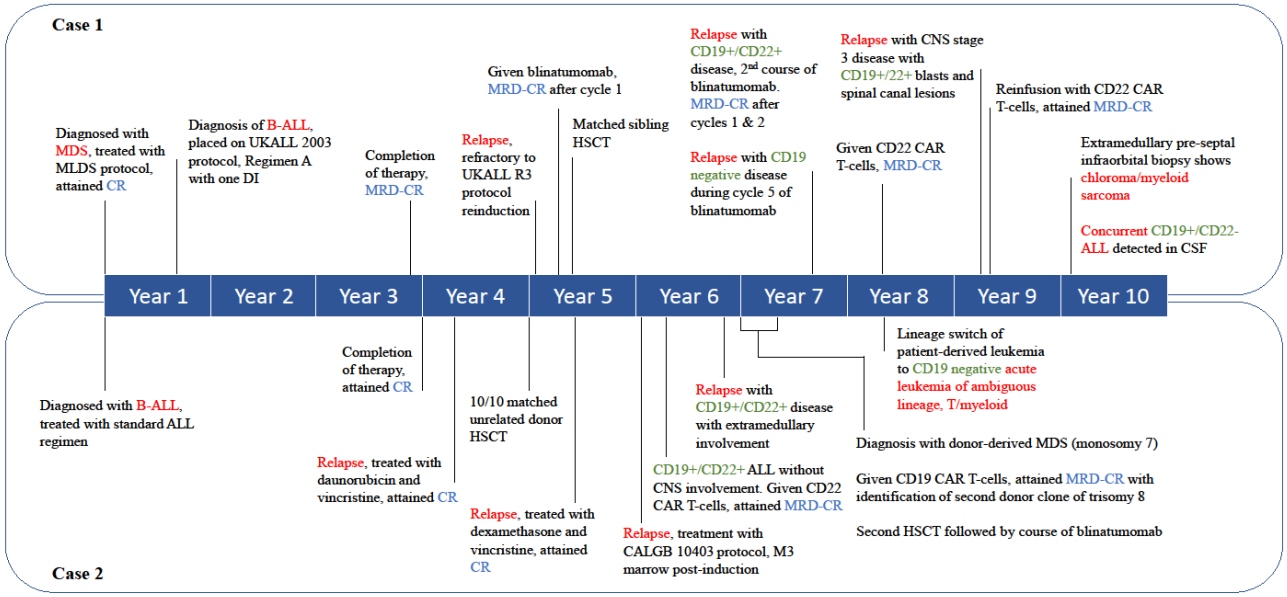
Primers:
Lentivector-Fwd specific primer: 5'CTGTTGTGTGACTCTGGTAACT3'
Lentivector probe: 5'/56-FAM/AAATCTCTA/ZEN/GCAGTGGCGCCCG/3IABkFQ/3'

Lentivector-Rev specific primer: 5′-TCGCTTTCAAGTCCCTGTTC-3′
MKL2 Fwd primer: 5'AGATCAGAAGGGTGAGAAGAATG3'
MKL2 Rev primer: 5'GGATGGTCTGGTAGTTGTAGTG3'

MKL2 probe: 5'-/5HEX/TGTTCCTGC/ZEN/AACTGCAGATCCTGA/3IABkFQ/-3'.

2.0. Figures

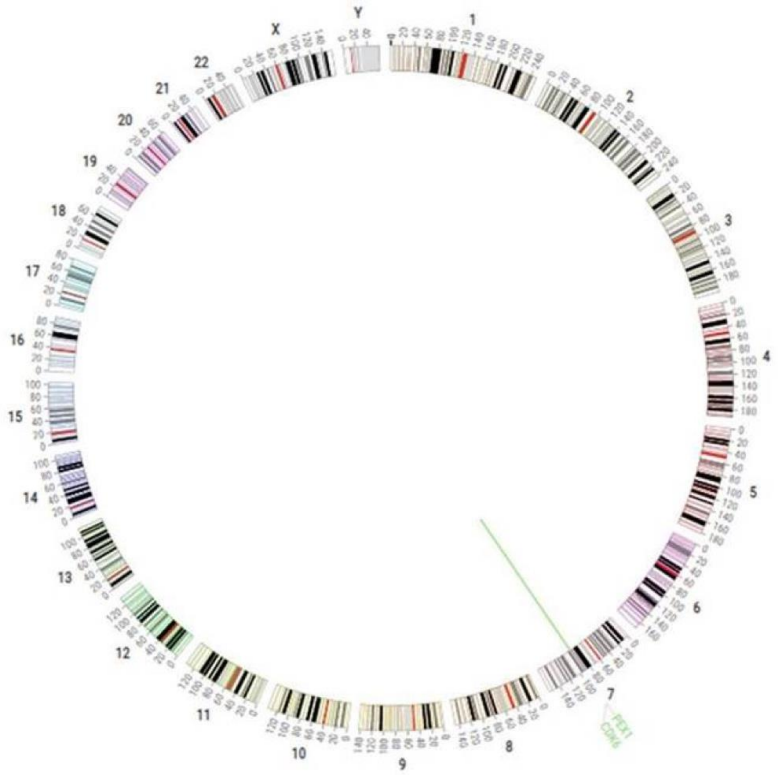
2.1. Timeline of clinical presentations

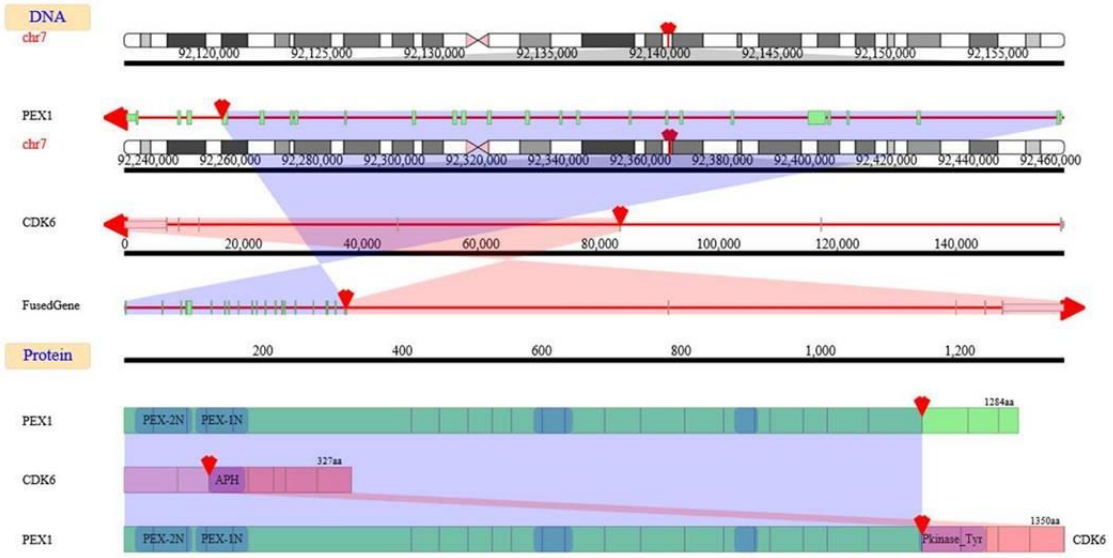


Abbreviations: MDS: myelodysplastic syndrome; MLDS: myeloid leukemia of Down Syndrome; CR: complete remission; ALL: acute lymphoblastic leukemia; B-ALL: B-cell ALL; MRD-CR: minimal residual disease negative CR; HSCT: hematopoietic stem cell transplant; CAR: chimeric antigen receptor; CNS: central nervous system

2.2. Identification of PEX1-CDK6 fusion in case 1

2.2.1.





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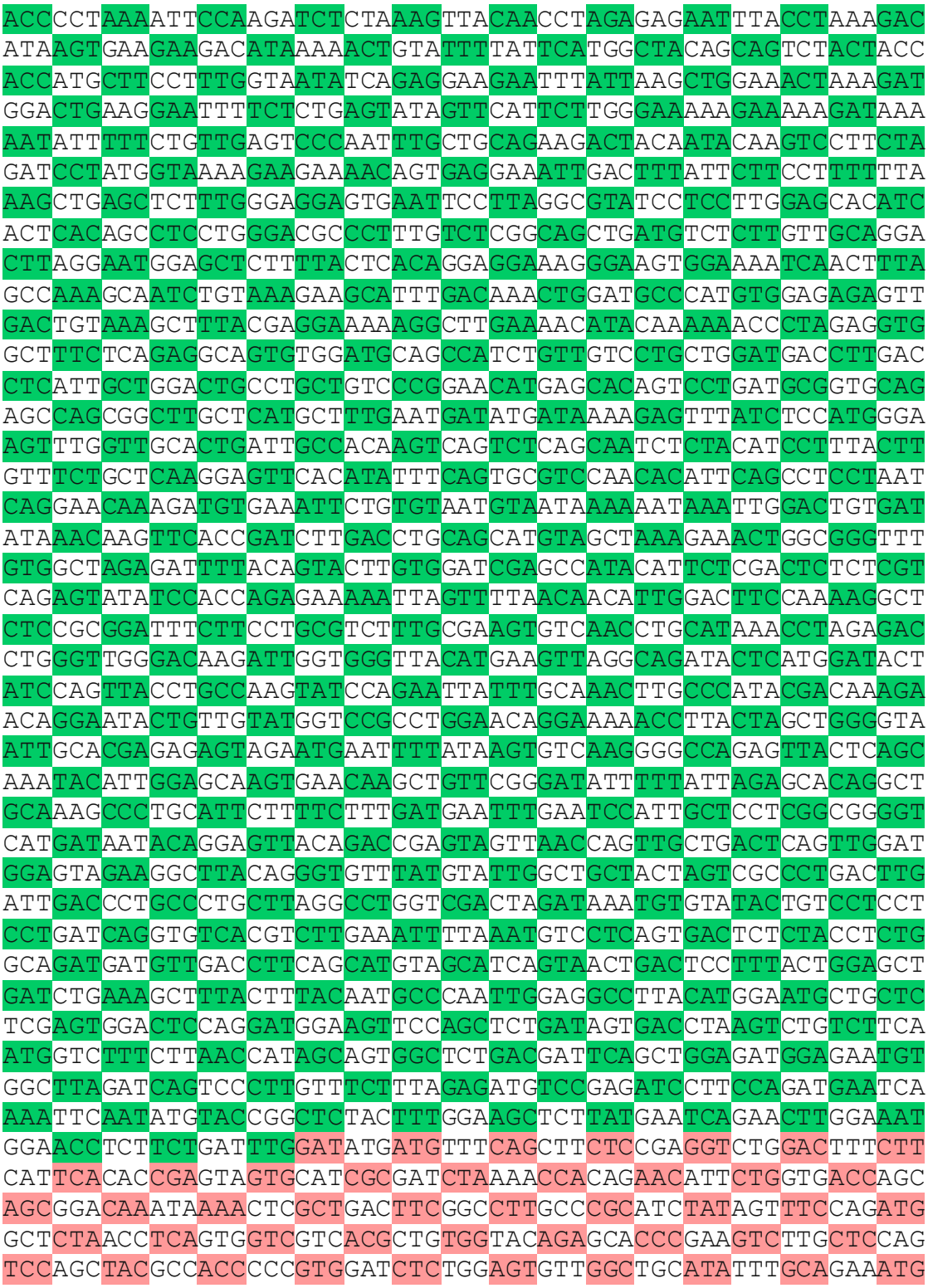

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TGA

Protein

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-DDWEILELHAVSLEQHLLDQIRIVFPKAIFPVWVDQQTYIFIQIVALIPAASYGRLE-
-TDTKLLIQP-KTRRAKENTFSKADAEYKKLHSYGRDQKGMMKELQTKQLQSNTVGITE
SNENESEIPVDSSSVASLWTMIGSIFSFQSEKKQETSWGLTEINAFKNMQSKVVPLD
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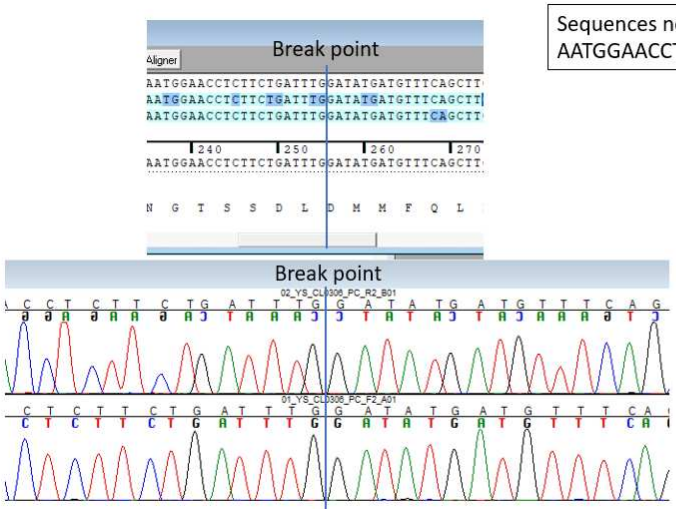
2.2.2. Sanger sequencing of PEX1-CDK6 fusion



Sequences near the break point:
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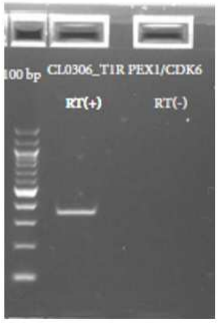

3.0. Tables

3.1. Chronological review of immunophenotypic and cytogenetic evaluation of disease

Case 1				
Months from Initial Diagnosis	**Diagnosis**	**Disease Sites**	**Immunophenotype**	**Cytogenetics**
At Diagnosis	MDS	Bone marrow	N/A	Normal by conventional karyotyping
8	B-ALL	Bone marrow	N/A	t(5;15)(q3;q15) FISH showed relocation of intact *PDGFRB* from 5q to 15q, consistent with the t(5;15), but with no *PDGFRB* rearrangement. There was no evidence of *PDGFRA*, *PDGFRB* or *FGFR1* gene rearrangements by interphase FISH.
80	B-ALL (2nd relapse, post-HSCT)	Bone marrow	CD45 (>95%), **CD19 (89%)**, CD10 (81%), **CD22 (90%)**, CD20 (41%), TdT (88%), CD33 (9%), CD34 (10%), CD10 (81%), CD117 (3%), CD14 (7%), CD13 (7%), CD2 (2%), CD4 (7%), CD3 (<1%), CD8 (8%), CD64 (6%), HLA-DR (93%), CD7 (<1%), MPO (7%)	45~49,XY,2,add(2)(p16),add(3)(q12),t(5;15)(q3;q15),+6,add(8)(p1),9,l(9)(q10),+11,add(11)(p1),add(12)(p1),add(16)(p13),add(16)(p13),add(16)(p13),add(17)(p1),add(19)(q1),+21c,+2~3mar[cp5]//46,XX[7]
87	B-ALL (3rd relapse, post-second round of blinatumomab)	Bone marrow, left temporal bone	**CD22 (>99%)**, CD10, bright CD24, moderate CD38, dim CD45, intracellular CD79a, dim CD20, dim CD81 **CD19 negative**, CD34/CD15/CD123 negative	N/A
99	B-ALL (post-first round of CD22 CAR therapy)	Paraspinal/spinal canal lesions	**Bright CD19 (100%)**, **CD22 (>99% positive)**, moderate to bright CD10,	N/A

		(T10-12, L4-L5), CSF	CD38, partial CD24 (38% positive), dim CD45 CD34 negative	
110	B-ALL (post-second round of CD22 CAR therapy) AML (myeloid sarcoma)	ALL: Central spinal cord (C7, T8, L1, L3), CSF AML: left inferior peri-orbital soft tissue	ALL: **CD19 positive**, moderately dim CD38, partial CD45 **CD22 negative**, CD10/CD11b/ CD13/CD14/CD15/CD24/ CD34/CD36/CD64 negative AML: moderate CD45, bright HLA-DR, CD38, dim to negative CD13, dim CD33, MPO positive **CD19 negative, CD22 negative**, CD3/CD10/CD14/CD16/CD24/CD34/ CD56/CD66b/CD79a/CD117 negative, TdT negative	*PEX1-CDK6* fusion (identified by RNAseq, confirmed by Sanger sequencing)
Case 2				
Months from Initial Diagnosis	**Diagnosis**	**Disease Sites**	**Immunophenotype**	**Cytogenetics**
At Diagnosis	B-ALL	Bone marrow	N/A	N/A
63	B-ALL (3^{nd} relapse)	Bone marrow	**CD19 positive (>99%)**, **CD22 positive (99%)**, CD24, bright CD10, dim to negative CD34, dim to negative CD38, dim CD45, bright CD13 CD3/CD14/CD16/CD56 negative	45,X,-X,add(4)(p14),der(8;12)(q10;q10),?add(9)(p13),-16,?add(21)(q22),+2mar[12]/46,XY[8] Positive for loss of the *CDKN2A* locus (also known as p16) in 91.5% of cells. Positive for a translocation between chromosomes 12 and 21 in 99.5% of cells (*ETV6-RUNX1*). Negative for a translocation between chromosomes 9 and 22.

				Negative for loss or rearrangement of 11q23. ISCN: 46,XY,?inv(17)(p11.2q11.2) or ?dup(17)(q11.2) or ?ins(17;?)(q11.2;?)[3]/46,XY[17] nuc ish(DXZ1x1)[3/507]/(DXZ1x2)[6/507]//(DXZ1,DYZ1)x1[498/507]
70	B-ALL (Relapse post-CD22 CAR therapy)	Bone marrow, pancreatic head, spleen, mesentery, medial left breast	**CD19 positive (100%)**, **CD22 positive (100%)**, bright CD24, bright CD10, spectrum of CD34 from moderate to negative (predominantly negative), dim CD38, dim CD45, bright CD13 CD3/CD14/CD16/CD20/CD56 negative	N/A
72	B-ALL (Prior to CD19 CAR therapy) Donor-derived MDS	Bone marrow, bilateral breasts, bilateral ovaries, parietal bone, skeletal involvement	**CD19 positive**, CD10, CD33 (majority positive, dim), CD38, CD45 (dim), CD58, CD71 CD2/CD13/CD14/CD20/CD34 negative	45,XY,-7[10] //46,XY[10] ISCN FISH result: ish t(12;21)(p13;q22)[10/25]//-7(D7Z1-,D7S486-)[9/24].nuc ish(ETV6x2,RUNX1x3)(ETV6 con RUNX1x1)[51/100]//(D7Z1,D7S486)x1[6/100] Summary: There are two unrelated abnormal clones in the current bone marrow specimen. The clone with the *ETV6-RUNX1* rearrangement from this patient's B-cell leukemia is still present. In addition, a clone with monosomy 7 is now present in cells of donor origin.
88	Acute leukemia of ambiguous lineage (T/myeloid, post-	Bone marrow, bilateral axilla/breasts	CD3 positive (cytoplasmic), CD33, CD34, CD117, CD133, MPO (subset)	Post-CD19 CAR cytogenetics:

	CD19 CAR therapy and HSCT)		**CD19 negative**, CD5/CD11c/CD14/CD13/CD123 negative	ISCN result: //46,X,-Y,+8[5] //45,XY,-7[5] //46,XY[10] Result Summary: There are two abnormal clones present in cells of donor origin: one with monosomy 7 and one with trisomy 8 and loss of Y. Relapse post-HSCT cytogenetics: Cytogenetics: 2 populations Patient derived - *ETV6-RUNX1* Patient derived - *ETV6-RUNX1* with delXq
Abbreviations: MDS: myelodysplastic syndrome; ALL: acute lymphoblastic leukemia; B-ALL: B cell ALL; FISH: fluorescence in situ hybridization; HSCT: hematopoietic stem cell transplant: CAR: chimeric antigen receptor; CSF: cerebrospinal fluid; AML: acute myeloid leukemia; N/A: not available				