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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. CART-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), 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. 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 CART-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

Case 1
- Diagnosed with MDS, treated with MLDS protocol, attained CR
- Diagnosis of B-ALL, placed on UKALL 2003 protocol, Regimen A with one DS
- Completion of therapy, MRD-CR
- Relapse, refractory to UKALL R3 protocol readmission
- Matched sibling HSCT
- Given blinatumomab, MDS-CR after cycle 1
- Relapse with CD19+/CD22+ disease, 2nd course of blinatumomab, MDS-CR after cycles 1 & 2
- Relapse with CD19 negative disease during cycle 5 of blinatumomab
- Given CD22 CAR T-cells, MDS-CR
- Relapse with CNS stage 3 disease with CD19+/22+ blasts and spinal cord lesions
- Relapse with CD22 CAR T-cells, attained MRD-CR
- Extramedullary pre-septal intracranial biopsy shows B-ACROSS/myeloid sarcoma
- Concomitant CD19+/CD22- ALL detected in CSF

Case 2
- Diagnosed with B-ALL, treated with standard ALL regimens
- Relapse, treated with daunorubicin and vincristine, attained CR
- Relapse, treated with daunorubicin and vincristine, attained CR
- 10/10 matched unrelated donor HSCT
- Relapse with CD19+/CD22+ disease with extramedullary involvement
- CD19+/CD22+ ALL without CNS involvement. Given CD22 CAR T-cells, attained MRD-CR
- Relapse, treatment with CALGB 10403 protocol, M3 narrow post-induction
- Lineage switch of patient-derived leukemia to CD19 negative acute leukemia of ambiguous lineage, T-cell
- Diagnosis of donor-derived MDS (monosomy 7)
- Given CD19 CAR T-cells, attained MRD-CR with identification of second donor clone of t(8;14) R
- Second HSCT followed by course of blinatumomab

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.
TTTCGTAGAAAGCCTCTTTTTCGTGGAAGTTCAGATGTTGATCAACTAGGAAAAATC
TTGGACGTGATTGGACTCCCAGGAGAAGAAGACTGGCCTAGAGATGTTGCCCTTCCC
AGGCAGGCTTTTCATTCAAAATCTGCCCAACCAATTGAGAAGTTTGTAACAGATATC
GATGAACTAGGCAAAGACCTACTTCTGAAGTGTTTGACATTTAACCCAGCCAAAAGA
ATATCTGCCTACAGTGCCCTGTCTCACCCATACTTCCAGGACCTGGAAAGGTGCAAA
GAAAACCTGGATTCCCACCTGCCGCCCAGCCAGAACACCTCGGAGCTGAATACAGCT
G

Protein

MWGSRLAGAGGGAAVTVAFTNARDCFLHLPRRLVAQLHLLQNQAIEVVWSHQPAFLS
WVEGRHFSDQGENVAEINRQVGQKLGLSNGGQVFLKPCSQVHYLQVHEVQKLRPL
QKQSKTKQNLSPEKEQKMEPLDDQKKIRSDHNEEDEAKCVALQVWNGLEELNNAI
KYTKNVEVHLKGVWIPDDLRLKRNLINEMHAVVRITPVEVTPIKPRSLKLQPRENLPK
DISEEDKITVYFYSWLQSQSTTMLPLVISEEEFILKETKDLKEFSLISVHSEKEKD
KNIPLLSPNLLQKTTIQVLLPMVEENELFEDIPFLKLSLGLVNSLVQSGEHE
ITHSLLGRPLSLQSMSSLVAGLRNGALLLITGKSGKSTLAIAKCEAFDKLDHAHVER
RQCGAENILEAQEEVEAFSEAVWMQPVSVVLDLDDLIAGLPLAVEPEHESPDAD
QSRLHAHALNMDIEMFISHGSLVALIATSQSQSLPHLVSQAQVHIFCQVHQP
NQEQRCIEILCNVIKKNLDICINFTDLIDLQHVAETKGFEVFARDFTVLVDRAISHRLS
RQSI5TREKVLITLDFQ5KARGLPASLSRSLNHLKFRDLGWDIKGLHEVQILMD
TQILPACYPELFANLPIRTGTLLYGEFCGKTLAGYITARESRMNFTSWKCEPLL
SKYIGASEQAVRDFIFIRAQAAPKCIFFDFESESIAJORHGDNTGTVDVQNLTTQL
DGVEQLQGYVIAATSRPDLIDAPRLPRLDLCVYPDDQVSRLEILNVLSDSLP
LADDVQHVASVTSFDGALDIALNYAQLEALHCMLSSGLQDSSDSDSLGL
SMVFLNHSSSGSSDSAGDCEGCLDQSLVSLEMSIPEDESKFNMYRLFGSSYSELG
NGTSDDFMPOULRGLDPEHLHSHRVVHRLKPQINLVTSOGIJKLADFGLARITYCEF
MALTSSVVTLYRRAEVLQSSYATVPLDSVGFCIFCFRKRPLFRGSSDVQLGK
ILDVIGLPEDDFRDPVDDFLQPAFHSKSAQQPIEFVTDILELGKDLKLKCLTFNP
KRISAYALSHPYFQDLERCKENLDSLPHPSQNTSLENLTA*

2.2.2. Sanger sequencing of PEX1-CDK6 fusion
Sequences near the break point:
AATGGAACCTCTTCTGATTAGGATATGATGTFTCAGCCTT
### 3.0. Tables

#### 3.1. Chronological review of immunophenotypic and cytogenetic evaluation of disease

<table>
<thead>
<tr>
<th>Case 1</th>
<th>Months from Initial Diagnosis</th>
<th>Diagnosis</th>
<th>Disease Sites</th>
<th>Immunophenotype</th>
<th>Cytogenetics</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>At Diagnosis</td>
<td>MDS</td>
<td>Bone marrow</td>
<td>N/A</td>
<td>Normal by conventional karyotyping</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>B-ALL</td>
<td>Bone marrow</td>
<td>N/A</td>
<td>t(5;15)(q3;q15)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
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<td></td>
<td>FISH showed relocation of intact <em>PDGFB</em> from 5q to 15q, consistent with the t(5;15), but with no <em>PDGFB</em> rearrangement. There was no evidence of <em>PDGFA</em>, <em>PDGFB</em> or <em>FGFR1</em> gene rearrangements by interphase FISH.</td>
</tr>
<tr>
<td></td>
<td>80</td>
<td>B-ALL (2nd relapse, post-HSCT)</td>
<td>Bone marrow</td>
<td>CD45 (&gt;95%), <strong>CD19</strong> (89%), CD10 (81%), <strong>CD22</strong> (90%), CD20 (41%), TdT (88%), CD33 (9%), CD34 (10%), CD10 (81%), CD117 (3%), CD14 (7%), CD13 (7%), CD2 (2%), CD4 (7%), CD3 (&lt;1%), CD8 (8%), CD64 (6%), HLA-DR (93%), CD7 (&lt;1%), MPO (7%)</td>
<td>45<del>49,XY,2,add(2)(p16),add(3)(q12),t(5;15)(q3;q15),+6,add(8)(p1),9,9(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</del>3mar[cp5]/46,XX[7]</td>
</tr>
<tr>
<td></td>
<td>87</td>
<td>B-ALL (3rd relapse, post-second round of blinatumomab)</td>
<td>Bone marrow, left temporal bone</td>
<td><strong>CD22</strong> (&gt;99%), CD10, bright CD24, moderate CD38, dim CD45, intracellular CD79a, dim CD20, dim CD81</td>
<td>N/A</td>
</tr>
<tr>
<td></td>
<td>99</td>
<td>B-ALL (post-first round of CD22 CAR therapy)</td>
<td>Paraspinal/spinal canal lesions</td>
<td><strong>Bright CD19</strong> (100%), <strong>CD22</strong> (&gt;99% positive), moderate to bright CD10,</td>
<td>N/A</td>
</tr>
<tr>
<td>Months from Initial Diagnosis</td>
<td>Diagnosis</td>
<td>Disease Sites</td>
<td>Immunophenotype</td>
<td>Cytogenetics</td>
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<td></td>
</tr>
<tr>
<td><strong>At Diagnosis</strong></td>
<td>B-ALL</td>
<td>Bone marrow</td>
<td>N/A</td>
<td>N/A</td>
<td></td>
</tr>
<tr>
<td><strong>63</strong></td>
<td>B-ALL (3rd relapse)</td>
<td>Bone marrow</td>
<td><strong>CD19 positive (&gt;99%), CD22 positive (99%)</strong>, CD24, bright CD10, dim to negative CD34, dim to negative CD38, dim CD45, bright CD13</td>
<td>45.X,-X,add(4)(p14),der(8;12)(q10;q10),?add(9)(p13),-16,?add(21)(q22),+2mar[12]/46,XY[8]</td>
<td></td>
</tr>
</tbody>
</table>
|                              |           |              | **CD3/CD14/CD16/CD56 negative** | 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.  

**Case 2**

B-ALL (post-second round of CD22 CAR therapy)  
AML (myeloid sarcoma)  

- **(T10-12, L4-L5), CSF**  
  - **CD38, partial CD24 (38% positive), dim CD45**  
  - **CD34 negative**  

- **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)
### B-ALL (Relapse post-CD22 CAR therapy)

<table>
<thead>
<tr>
<th>Patient ID</th>
<th>Diagnosis</th>
<th>Cytogenetics</th>
<th>Immunophenotype</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>70</td>
<td>B-ALL</td>
<td>46,XY,?inv(17)(p11.2q11.2) or ?dup(17)(q11.2) or ?ins(17;?)q11.2;?)3/46,XY17</td>
<td>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</td>
<td>N/A</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CD3/CD14/CD16/CD20/CD56 negative</td>
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</table>

### B-ALL (Prior to CD19 CAR therapy)

<table>
<thead>
<tr>
<th>Patient ID</th>
<th>Diagnosis</th>
<th>Cytogenetics</th>
<th>Immunophenotype</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>72</td>
<td>B-ALL</td>
<td>45,XY,-7[10] //46,XY[10]</td>
<td>CD19 positive, CD10, CD33 (majority positive, dim), CD38, CD45 (dim), CD58, CD71</td>
<td>N/A</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CD2/CD13/CD14/CD20/CD34 negative</td>
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</table>

### Acute leukemia of ambiguous lineage (T/myeloid, post-)

<table>
<thead>
<tr>
<th>Patient ID</th>
<th>Diagnosis</th>
<th>Cytogenetics</th>
<th>Immunophenotype</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>88</td>
<td>Acute leukemia</td>
<td>CD3 positive (cytoplasmic), CD33, CD34, CD117, CD133, MPO (subset)</td>
<td>Post-CD19 CAR cytogenetics:</td>
<td></td>
</tr>
</tbody>
</table>

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.
<table>
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<tr>
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<tbody>
<tr>
<td>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.</td>
<td></td>
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<tr>
<td>Relapse post-HSCT cytogenetics:</td>
<td></td>
<td></td>
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<tr>
<td>Cytogenetics: 2 populations</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patient derived - <em>ETV6-RUNX1</em></td>
<td></td>
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<tr>
<td>Patient derived - <em>ETV6-RUNX1</em> with delXq</td>
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</tr>
</tbody>
</table>

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