DR. GEORGINA L RYLAND (Orcid ID : 0000-0002-4990-0961)

DR. PIERS ALEXANDER BLOMBERY (Orcid ID : 0000-0002-9902-0022)

Article type : Letters

Inotuzumab ozogamicin resistance associated with a novel *CD22* truncating mutation in a case of B-acute lymphoblastic leukaemia

Georgina L. Ryland[1,2,3], Allison Barraclough[4], Chun Yew Fong[4,5], Shaun Fleming[6], Ashish Bajel[7], Oliver Hofmann[2], David Westerman[1,3,7], Sean Grimmond[2] and Piers Blombery[1,3,7]

[1]Department of Pathology, Peter MacCallum Cancer Centre, Melbourne, Australia

[2]Centre for Cancer Research, University of Melbourne, Melbourne, Australia

[3]Sir Peter MacCallum Department of Oncology, University of Melbourne, Melbourne, Australia

[4]Department of Clinical Haematology, Austin Health, Heidelberg, Australia

[5]Olivia Newton John Cancer Research Institute, Heidelberg, Australia

[6]Department of Clinical Haematology, Alfred Hospital, Melbourne, Australia

[7]Clinical Haematology, Peter MacCallum Cancer Centre and Royal Melbourne Hospital, Melbourne, Australia

Running title: Novel CD22 therapy resistance mutation in B-ALL

Correspondence: Georgina Ryland, Department of Pathology, Peter MacCallum Cancer Centre, 305 Grattan Street, Melbourne, Victoria 3000, Australia; email: georgina.ryland@petermac.org.

Relapsed B-acute lymphoblastic leukaemia (B-ALL) remains a difficult disease to treat and is associated with a poor prognosis. Antibody-based immunotherapy (blinatumomab), antibody-drug conjugates (inotuzumab ozogamicin, InO) and cellular immunotherapies (chimeric antigen receptor T cells, CAR-T) have emerged as effective treatment options in patients with relapsed disease(1-3). Whilst genomic sequencing studies have uncovered mechanisms of intrinsic and acquired therapy resistance, a significant proportion of clinical resistance remains to be understood. Here, we describe a patient with relapsed B-ALL who acquired a novel *CD22* mutation predicted to result in premature truncation of CD22 arising under selective pressure of InO, an anti-CD22 targeted therapy.

A 17-year-old man presented with anaemia (85g/L) and leukocytosis ($68.4x10^9$/L) after several weeks of abdominal fullness, lethargy, light headedness, night sweats, bruising and weight loss. Bone marrow biopsy demonstrated 97% blasts with an immunophenotype consistent with pre B-ALL. Chromosome analysis revealed a normal male karyotype. He was commenced on the FRALLE-93 protocol(4, 5) and had undetectable measurable residual disease (MRD) by flow cytometry at day 94 (sensitivity $<10^{-4}$). The patient received consolidation and maintenance as per protocol without major complication.

One month after completion of maintenance he relapsed with a bone marrow aspirate demonstrating 92% blasts with an immunophenotype and karyotype unchanged from diagnosis. He was treated with blinatumomab achieving a morphological remission with persistent MRD (0.15% of mononuclear cells). He was planned for allogeneic transplantation however after a single cycle of blinatumomab he had overt disease progression with a bone marrow aspirate demonstrating 51% blasts.

He was commenced on InO, achieving undetectable MRD after one cycle (sensitivity $<10^{-4}$), however after two cycles his disease progressed with 75% blasts in the bone marrow and an immunophenotype that was still consistent with pre B-ALL but was now notably negative for CD22. To understand potential mechanisms contributing to leukaemia progression following InO, we performed whole genome sequencing (WGS) and whole transcriptome RNA-sequencing (WTS) on this relapse sample collected 59 days after first administration of InO (R-InO) (see Supporting Information). Additional genomic findings in R-InO and from WGS-WTS performed at diagnosis and progression post CAR-T are summarized in the Supporting Information (Fig S1A-E, Table S1).

WGS identified a truncating mutation in exon 4 of *CD22* (NM_001771.3:c.712_713insCT; p.(Val238Alafs*2)) (Fig 1A), the transmembrane glycoprotein target of InO, an anti-CD22 monoclonal

antibody conjugated to the DNA-binding cytotoxic calicheamicin that is rapidly internalized to deliver calicheamicin within the cytoplasmic compartment. This mutation was homozygous, occurring within a region of copy neutral loss of heterozygosity on chromosome 19q (Fig S1B) and was present in a high proportion of the cancer cell fraction given a variant allele frequency (VAF) of 51.1% in a sample with an estimated tumour purity of 56%. The site of the mutation in *CD22* (valine 238) occurs between the second (d2) and third (d3) extracellular immunoglobulin(Ig)-like domains, inducing a frameshift in the CD22 mRNA sequence that is predicted to result in an aberrant reading frame introducing a premature stop codon after an additional two amino acids and prior to the downstream Ig-like domains (d3-d7), transmembrane domain and the cytoplasmic immunoreceptor tyrosine-based inhibitory motif (ITIM) which normally mediates CD22 activity through recruitment of inhibitory phosphatases that facilitate dephosphorylation of stimulatory co-receptors(6). The observation that 99.6% of leukemic blasts were now CD22 negative by flow cytometry (Fig 1B) is consistent with this truncating mutation leading to loss of surface CD22 expression on leukemic blasts. WTS performed on the R-InO sample demonstrated expression of the mutant allele suggesting at least partial avoidance of nonsense mediated decay (Fig S1D).

In order to investigate when the *CD22* mutation emerged in this patient, we performed sensitive allele specific droplet digital PCR (ddPCR) for the *CD22* Val238Alafs*2 mutation using the Bio-Rad Droplet Digital PCR system (Bio-Rad, Hercules, CA) (Fig 1B). The *CD22* Val238Alafs*2 mutation was detected at 49.0% VAF in R-InO, consistent with the allele fraction estimated by WGS, but was undetectable to an assay limit of detection of 0.01% in five prior bone marrow samples including three with high blast burden: diagnosis, first relapse post maintenance (where blasts were CD22 positive) and disease progression post blinatumomab.

Given the patients previous exposure to chemotherapy without durable remission and documented CD19 expression on leukemic blasts, CD19-directed CAR-T therapy (tisagenlecleucel) was planned and he underwent autologous lymphocyte collection by apheresis. He received bridging chemotherapy with cyclophosphamide, doxorubicin, vincristine and prednisolone achieving transient disease control with MRD detectable in the marrow (0.21% of white blood cells), followed by overt progression with 39.2% blasts. He underwent lymphodepletion and CAR-T infusion however had rapid disease progression at day 8 post-infusion and died of progressive disease 30 days post CAR-T infusion. Flow cytometry performed just prior to and after CAR-T therapy demonstrated re-establishment of normal CD22 expression on leukemic blasts. We performed ddPCR on these samples and demonstrated regression of the *CD22* mutated clone (VAF 2.1% and 0.1% respectively) (Fig 1B). The correlation over

time of the *CD22* mutant clone quantified by ddPCR with CD22 expression by flow cytometry is consistent with (i) the lack of CD22 expression being a direct result of the truncating *CD22* mutation and (ii) the selective advantage of the CD22 mutant leukemic clone existing only in the presence of CD22-directed therapy.

The restricted expression on B cells along with the rapid internalization upon antigen binding has made CD22 an attractive target for the delivery of antibody conjugated toxins such as InO, and more recently, a target for CAR-T therapy(2, 7, 8). Decreased surface CD22 expression and receptor density have been reported as mechanisms of acquired resistance to InO(9-11) and CD22 CAR-T(7, 12) in some patients relapsing with B-lineage acute leukaemia (i.e. non-lineage switch). This is similar to CD19 antigen modulation as a mechanism of escape from CD19-directed therapies such as blinatumomab and CD19 CAR-T, where mutation or isoform switching can contribute to CD19 antigen loss(13-15). Post-transcriptional (rather than genomic) mechanisms downregulating CD22 protein expression on leukemic blasts have been postulated as the cause of CD22-targeted therapy resistance, however these observations are based on investigations in only a small number of patients(7, 10). Our finding of a novel truncating *CD22* mutation with concomitant loss of the receptor from the cell surface represents a new mechanism of leukemic cell escape not previously reported in patients treated with CD22-directed therapies and should be considered in patients who relapse with CD22 negative disease. Interestingly, in addition to the truncating *CD22* mutation our patient had emergence of multiple other genomic resistance mechanisms to therapies received including mutations in *NT5C2* and *ABCB1* (Supporting Information) consistent with the paradigm of the polyclonal nature of therapeutic resistance. Our observation adds to the growing list of resistance mechanisms emerging in response to antigen-directed therapy in B-ALL, and highlights the need for an improved understanding of how optimal combinations and timing of both cytotoxic and antigen-directed therapy can be best implemented to circumvent leukemic cell escape.

Acknowledgments

This work was supported by grants from the Wilson Centre for Lymphoma Genomics and the Snowdome Foundation.

Author contributions

G.R. and P.B. analysed data and wrote the manuscript; A. Barraclough, C.Y.F., S.F. and A. Bajel decided on critical clinical issues regarding patient care; O.H., D.W. and S.G. were involved in interpretation of pathology and genomic data; and all authors discussed and approved the final manuscript.

Conflicts of interest

A. Barraclough has received travel support from Roche; C.Y.F. has served on the Speakers' Bureau for Amgen and Pfizer and has served on the Scientific Advisory Board for Amgen and Pfizer. C.Y.F. has received research funding from Amgen unrelated to the current manuscript. All other authors declare no competing financial interests.

References

1. Kantarjian H, Stein A, Gokbuget N, Fielding AK, Schuh AC, Ribera JM, et al. Blinatumomab versus Chemotherapy for Advanced Acute Lymphoblastic Leukemia. N Engl J Med. 2017;376(9):836-47.
2. Kantarjian HM, DeAngelo DJ, Stelljes M, Martinelli G, Liedtke M, Stock W, et al. Inotuzumab Ozogamicin versus Standard Therapy for Acute Lymphoblastic Leukemia. N Engl J Med. 2016;375(8):740-53.
3. Maude SL, Laetsch TW, Buechner J, Rives S, Boyer M, Bittencourt H, et al. Tisagenlecleucel in Children and Young Adults with B-Cell Lymphoblastic Leukemia. N Engl J Med. 2018;378(5):439-48.
4. Hocking J, Schwarer AP, Gasiorowski R, Patil S, Avery S, Gibson J, et al. Excellent outcomes for adolescents and adults with acute lymphoblastic leukemia and lymphoma without allogeneic stem cell transplant: the FRALLE-93 pediatric protocol. Leukemia & lymphoma. 2014;55(12):2801-7.
5. Boissel N, Auclerc MF, Lheritier V, Perel Y, Thomas X, Leblanc T, et al. Should adolescents with acute lymphoblastic leukemia be treated as old children or young adults? Comparison of the French FRALLE-93 and LALA-94 trials. J Clin Oncol. 2003;21(5):774-80.
6. Nitschke L. The role of CD22 and other inhibitory co-receptors in B-cell activation. Curr Opin Immunol. 2005;17(3):290-7.
7. Fry TJ, Shah NN, Orentas RJ, Stetler-Stevenson M, Yuan CM, Ramakrishna S, et al. CD22-targeted CAR T cells induce remission in B-ALL that is naive or resistant to CD19-targeted CAR immunotherapy. Nat Med. 2018;24(1):20-8.
8. DeAngelo DJ, Stock W, Stein AS, Shustov A, Liedtke M, Schiffer CA, et al. Inotuzumab ozogamicin in adults with relapsed or refractory CD22-positive acute lymphoblastic leukemia: a phase 1/2 study. Blood Adv. 2017;1(15):1167-80.
9. Kantarjian HM, Stock W, Cassaday RD, DeAngelo DJ, Jabbour EJ, O'Brien SM, et al. Comparison of CD22 Expression between Baseline, End of Treatment, and Relapse Among Patients Treated with Inotuzumab Ozogamicin Who Responded and Subsequently Relapsed in Two Clinical Trials. Blood. 2018;132(Supplement 1):2699-.

10. Paul MR, Wong V, Aristizabal P, Kuo DJ. Treatment of Recurrent Refractory Pediatric Pre-B Acute Lymphoblastic Leukemia Using Inotuzumab Ozogamicin Monotherapy Resulting in CD22 Antigen Expression Loss as a Mechanism of Therapy Resistance. J Pediatr Hematol Oncol. 2019;41(8):e546-e9.

11. Bhojwani D, Sposto R, Shah NN, Rodriguez V, Yuan C, Stetler-Stevenson M, et al. Inotuzumab ozogamicin in pediatric patients with relapsed/refractory acute lymphoblastic leukemia. Leukemia. 2019;33(4):884-92.

12. Yates B, Shalabi H, Salem D, Delbrook C, Yuan CM, Stetler-Stevenson M, et al. Sequential CD22 Targeting Impacts CD22 CAR-T Cell Response. Blood. 2018;132(Supplement 1):282-.

13. Sotillo E, Barrett DM, Black KL, Bagashev A, Oldridge D, Wu G, et al. Convergence of Acquired Mutations and Alternative Splicing of CD19 Enables Resistance to CART-19 Immunotherapy. Cancer Discov. 2015;5(12):1282-95.

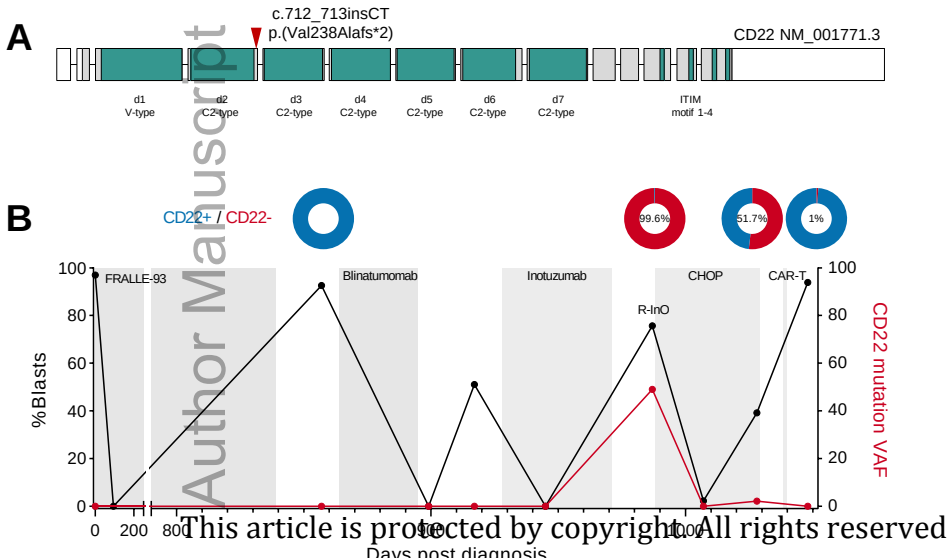
14. Topp MS, Gokbuget N, Zugmaier G, Klappers P, Stelljes M, Neumann S, et al. Phase II trial of the anti-CD19 bispecific T cell-engager blinatumomab shows hematologic and molecular remissions in patients with relapsed or refractory B-precursor acute lymphoblastic leukemia. J Clin Oncol. 2014;32(36):4134-40.

15. Orlando EJ, Han X, Tribouley C, Wood PA, Leary RJ, Riester M, et al. Genetic mechanisms of target antigen loss in CAR19 therapy of acute lymphoblastic leukemia. Nat Med. 2018;24(10):1504-6.

Figure 1. A novel loss-of-function *CD22* mutation emergent after inotuzumab ozogamicin. (A) Schematic representation of *CD22* exons 1-14 including coding (grey) and non-coding (white) regions. The locations of the extracellular immunoglobulin domains (Ig-like V-type d1 and C2-type d2-d7) and the intracellular tyrosine-based inhibitory motifs (ITIM motif 1-4) are shown (green). The Val238Alafs*2 truncating mutation detected in this patient is indicated by the arrowhead. (B) Variant allele frequency (VAF) of the Val238Alafs*2 mutation by droplet digital PCR (red), along with the proportion of bone marrow lymphoblasts by morphology (black). The proportion of CD22 positive (blue) and CD22 negative (red) lymphoblasts as a proportion of total lymphoblasts by flow cytometry are shown in the circles above; numbers within the circles enumerate the proportion of CD22 negative lymphoblasts.

Figure 1

bjh_16949_f1.pdf



Author/s:
Ryland, GL; Barraclough, A; Fong, CY; Fleming, S; Bajel, A; Hofmann, O; Westerman, D; Grimmond, S; Blombery, P

Title:
Inotuzumab ozogamicin resistance associated with a novel CD22 truncating mutation in a case of B-acute lymphoblastic leukaemia

Date:
2020-07-09

Citation:
Ryland, G. L., Barraclough, A., Fong, C. Y., Fleming, S., Bajel, A., Hofmann, O., Westerman, D., Grimmond, S. & Blombery, P. (2020). Inotuzumab ozogamicin resistance associated with a novel CD22 truncating mutation in a case of B-acute lymphoblastic leukaemia. BRITISH JOURNAL OF HAEMATOLOGY, 191 (1), pp.123-126. https://doi.org/10.1111/bjh.16949.