DR. DAVID M ROSS (Orcid ID : 0000-0001-7171-2935)

PROF. TIMOTHY HUGHES (Orcid ID : 0000-0002-0910-3730)

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Lenalidomide maintenance treatment after imatinib discontinuation: results of a phase 1 clinical trial in chronic myeloid leukaemia

David M Ross,[1-4] Ilaria S Pagani,[2,4] Yazad D Irani,[2] Jade Clarson,[1,2] Tamara Leclercq,[2] Phuong Dang,[2] Jennifer McLean,[2] Verity A Saunders,[2] Lisa Carne,[1] John Reynolds,[5] David S Ritchie,[6,7] Deborah L White,[2] Susan Branford,[3,8,9] Timothy P Hughes,[1-4] Agnes SM Yong[2,4]

1. Department of Haematology and Bone Marrow Transplantation, Royal Adelaide Hospital, Adelaide, Australia
2. Cancer Theme, South Australian Health and Medical Research Institute, Adelaide, Australia
3. Centre for Cancer Biology, SA Pathology and University of South Australia, Adelaide, Australia
4. School of Medicine, Faculty of Health Sciences, University of Adelaide, Adelaide, Australia
5. Faculty of Medicine, Nursing and Health Sciences, Monash University, Melbourne, Australia
6. Department of Clinical Haematology, Peter MacCallum Cancer Centre and Royal Melbourne Hospital, Melbourne, Australia

7. Melbourne Medical School, University of Melbourne, Melbourne, Australia
8. Department of Genetic and Molecular Pathology, SA Pathology, Adelaide, Australia
9. School of Pharmacy and Medical Science, University of South Australia, Adelaide, Australia

Corresponding author:
Dr D M Ross
Department of Haematology, Royal Adelaide Hospital, Port Road, Adelaide, SA 5000, Australia
Telephone: +61 8 7074 2429
Facsimile: +61 8 8204 5231
Email: david.ross@sa.gov.au

ORCID information
DMR 0000-0001-7171-2935
ISP 0000-0002-3216-2966
YDI 0000-0002-2861-5774
DSR 0000-0001-7329-6605
ASMY 0000-0001-9452-1533

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Treatment-free remission (TFR) is achieved by 40-60% of chronic myeloid leukaemia (CML) patients who stop imatinib treatment after a sustained *BCR-ABL1* level ≤0.0032% (MR4.5).(Ross*, et al* 2018, Saussele*, et al* 2018) Patients who need to restart imatinib typically regain MR4.5 within 3 months, but may need to remain on treatment indefinitely. There are limited data on the outcomes of a second TFR attempt (TFR2).(Legros*, et al* 2017, Ross*, et al* 2018) Evidence from several studies suggests that immunological factors, particularly increased numbers of natural killer (NK) cells, may influence TFR outcomes.(Ilander*, et al* 2017, Imagawa*, et al* 2015, Rea*, et al* 2017) Lenalidomide increases T- and NK-cell proliferation and activation, and enhances NK cytotoxicity.(Davies*, et al* 2001) It is widely used for the treatment of myeloma, but is not approved for CML. We designed a Phase 1b clinical trial ('LENI'; ACTRN12615001169538) for patients planning a TFR2 attempt using lenalidomide to augment immune function.

This single centre non-randomized clinical trial was planned to enrol 20 CML patients in MR4.5 for ≥12 months on imatinib after previously having failed a TFR attempt. In the combination phase of the study, patients continued imatinib at the previously established dose and added lenalidomide 5 mg daily for one month, with escalation as tolerated to 10 mg daily for a further 5 months. If patients maintained MR4.5, imatinib was discontinued and lenalidomide maintenance treatment was continued for 6 months. Aspirin was used as thromboprophylaxis, following standard practice when lenalidomide is used for myeloma. Lenalidomide could be stopped earlier, and imatinib restarted, in the event of loss of major molecular response (MMR; *BCR-ABL1* >0.1%). Real-time quantitative reverse transcription polymerase chain reaction (RQ-PCR) monitoring was performed every 3 months during the combination phase, and monthly for 12 months after stopping imatinib together with highly sensitive, patient-specific *BCR-ABL1* DNA PCR.(Ross*, et al* 2010, Ross*, et al* 2018) Immunological studies were planned at baseline, 3 and 6 months during the combination phase, at 3 and 6 months during the lenalidomide maintenance phase, and at 3, 6 and 12 months after stopping lenalidomide.(Hughes*, et al* 2017) The primary endpoint was the safety of lenalidomide in combination with imatinib.

The study was closed prematurely on the recommendation of the safety monitoring committee after only 3 patients were enrolled (Table I), all of whom had previously participated in the Australasian Leukaemia and Lymphoma Group CML8 (TWISTER) study.(Ross*, et al* 2018) All patients had MR4.5 and undetectable *BCR-ABL1* mRNA at study entry (UMRD4.5). Patient 2 remained on lenalidomide 5 mg daily due to cytopenia during the first month of the combination phase. The other patients completed the combination phase at the planned lenalidomide dose of 10 mg daily. Patient 1 developed unprovoked pulmonary embolism 4 months after stopping imatinib, while on lenalidomide maintenance. Patient 2 developed right optic neuritis, presumed ischaemic, 3 weeks after stopping imatinib while on lenalidomide maintenance therapy, which was then stopped. Eight weeks later she developed pulmonary embolism. Patient 3 was then taken off lenalidomide maintenance pre-emptively. The only other adverse event of grade 2+ occurring in >1 patient was neutropenia. Following the discontinuation of lenalidomide there were two further serious adverse events: oesophageal bleeding whilst on anticoagulation (Patient 3) and squamous cell carcinoma of the skin 10 months after lenalidomide was stopped (Patient 2).

All 3 patients remained in continuous UMRD4.5 at last follow-up 20-26 months after stopping imatinib (Figure 1). In the French RE-STIM study, in which patients stopped imatinib for a second time without additional treatment, 12/45 patients maintained MR4.5

(27%), and loss of UMRD4.5 within 3 months at the first TFR attempt (TFR1) was significantly associated with a higher risk of failure at TFR2 (hazard ratio: 2.0).(Legros*, et al* 2017) Our patients had molecular relapse characteristics at TFR1 that were comparable to those of the RE-STIM patients: the two patients who did not lose MMR had a significant increase in *BCR-ABL1* mRNA within the first four months. However, our patients also had an exceptionally long duration of UMRD4.5 after TFR1 (versus a median of 24.5 months in RE-STIM), and duration of deep molecular response may itself affect the probability of successful tyrosine kinase inhibitor (TKI) discontinuation.(Saussele*, et al* 2018)

The combination of imatinib and low dose lenalidomide was well-tolerated, with no unexpected toxicity, but lenalidomide maintenance after stopping imatinib was associated with thrombotic adverse events in the first two patients, leading to closure of the study. No clinical trial to date has reported a significantly increased rate of thrombotic events following imatinib discontinuation. There has been no prior study using lenalidomide in CML, and both the primary safety endpoint and the protocol-defined stopping rules in LENI referred only to adverse events during the combination phase. When designing the study, we based our estimate of thrombosis risk on data from patients with del(5q) myelodysplastic syndrome, because this is also a myeloid malignancy, treated with a similar lenalidomide dose, and typically not combined with corticosteroids or other cytotoxic therapy. In a clinical trial involving 103 patients on lenalidomide 10 mg daily for a median of 2 years, there were three occurrences of deep vein thrombosis.(List*, et al* 2006) The timing of the events in the LENI study raises the possibility that imatinib discontinuation and concomitant lenalidomide treatment interacted to potentiate the thrombotic risk. It is possible that imatinib has an anti-thrombotic effect that is lost on discontinuation, or that imatinib withdrawal leads to a pro-inflammatory, prothrombotic state.

Lenalidomide did not reduce the level of *BCR-ABL1* (by DNA PCR), but was associated with an increase in markers of immunological reactivity (Figure 1). Most of these changes were transient and reverted by the end of the combination phase. All three patients remained in TFR at last follow-up, but the small number of patients enrolled precludes any definitive assessment of this efficacy of this approach. An immunomodulatory strategy might be worthy of further investigation if the safety concerns can be addressed by changes to the sequence of imatinib/lenalidomide discontinuation, more effective thromboprophylaxis, or the use of an alternative immunomodulatory agent. Most importantly, our experience highlights the necessity for any TFR clinical trial involving novel treatment to balance the risk of the novel

intervention against the potential benefits of TFR, and to incorporate careful monitoring for adverse events both during treatment and after TKI discontinuation.

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DMR designed the study and wrote the paper; ISP and YDI performed research and wrote the paper; TL, PD, JM, VAS, and LC performed research; JR, DSR, DLW, SB, TPH, and ASMY designed the study and wrote the paper.

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Table I. Patient characteristics at study entry

	Patient		
	1	2	3
Age (years)	64	69	58
Sex	Male	Female	Male
Years since diagnosis of CML	17.4	15.9	12.3
Days to relapse (TFR1)	97	55	99
Peak *BCR-ABL1* at molecular relapse (TFR1)	0.03%	1.75%	0.09%
Years of imatinib re-treatment	9.7	7.5	7.5
Years of MR4.5 since TFR1	9.4	6.8	7.2

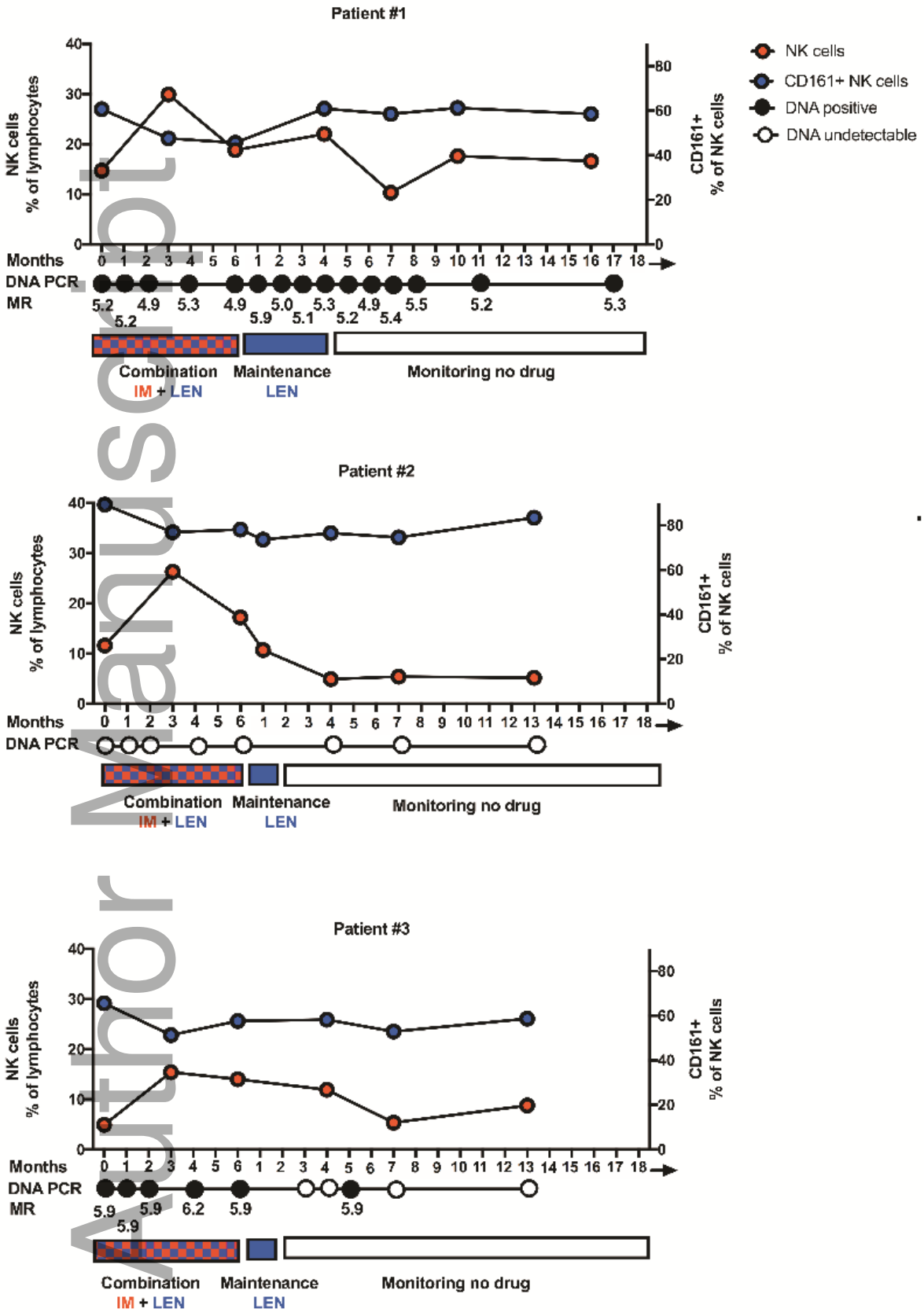
CML: chronic myeloid leukaemia; MR4.5: deep molecular response (*BCR-ABL1* level ≤0.0032%); TFR1: first attempt at first tyrosine kinase inhibitor discontinuation

Figure legend

Figure 1. Details of patient treatment, *BCR-ABL1* DNA, and NK cell proportions over time

Flow cytometry was used to characterize the phenotype and function of immune cells including natural killer (NK) cells, regulatory T cells (Tregs) and myeloid-derived suppressor cells (MDSC). Functional cytotoxic T-lymphocyte (CTL) responses to leukaemia-associated antigens (LAAs) WT1, BMI-1, PR3 and PRAME were assessed by interferon-γ (IFN-γ)/tumour-necrosis factor-α (TNF-α) FLUROSPOT using libraries of pentadecamer peptides overlapping by 11 amino acids spanning the entire protein. $CD3^{-}CD56^{+}$ NK cells (as a percentage of lymphocytes) increased significantly after commencing lenalidomide during the combination phase (median 11.6% at study entry vs 26.3% at 3 months, p=0.03), and gradually reduced to baseline over the course of study. Cytolytic $CD16^{+}$ NK cells, as a percentage of lymphocytes (left hand y axis), increased from 10.9% at baseline to 23.0% at 3 months (p=0.04), with a concomitant decrease of $CD161^{+}$ inhibitory receptor on NK cells (65.6% at baseline vs 51.3% at 3 months, p=0.004, right hand y axis). Paradoxically, the CD94/NKG2A inhibitory NK receptor and Tregs increased (41.1% at baseline vs 51.7% at 3 months, p=0.02; 4.8% at baseline vs 7.1% at 3 months, p=0.006, respectively) and reduced subsequently to baseline levels. LAA-CTL responses were observed in all three patients at baseline, and maintained in 77% of all subsequent time points tested, with peak responses during the combination phase. PRAME- and BMI-1-specific CTLs (predominantly IFN-γ and TNF-α driven, respectively) were the most abundant and were detected in all patients. LAA-CTL responses were detected up to 12 months after stopping lenalidomide. Months on

study are shown as baseline to 6 months of imatinib+lenalidomide combination treatment, then numbering re-starts from the discontinuation of imatinib. The results of *BCR-ABL1* DNA PCR are shown as $\log_{10}$ molecular response values) below the x axis. *BCR-ABL1* DNA was detected at study entry in Patients 1 and 3 with no significant change in the level over the course of the trial (median MR5.2, MR5.9), while Patient 2 had undetectable *BCR-ABL1* DNA in all tests (limit of detection, MR6.2). IM=imatinib; LEN=lenalidomide; MR=molecular response.



Author/s:
Ross, DM; Pagani, IS; Irani, YD; Clarson, J; Leclercq, T; Phuong, D; McLean, J; Saunders, VA; Carne, L; Reynolds, J; Ritchie, DS; White, DL; Branford, S; Hughes, TP; Yong, ASM

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