Supplemental materials for

BCR-ABL GENE EXPRESSION IS REQUIRED FOR ITS MUTATIONS IN A NOVEL KCL-22 CELL CULTURE MODEL FOR ACQUIRED RESISTANCE OF CHRONIC MYELOGENOUS LEUKEMIA

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Running head: BCR-ABL mutations in a culture model

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SUPPLEMENTAL METHODS:

Real-time PCR for gene amplification and expression analysis

Quantitative real-time PCR was performed with SuperScript III platinum two-step qRT-PCR kit with SYBR Green (Invitrogen) as per the manufacture's instruction on the BioRad machine OPTICON. For BCR-ABL RNA analysis, we extracted total cellular RNA with Trizol (Invitrogen) using a standard protocol. The primer pairs for ABL genomic DNA analysis were 5'- GCCTGTCTCTGTGGGCTGAAG-3' and 5'-CAAGGCGTCTGCTGGCATTA-3'; primers for BCR-ABL RNA analysis were 5'-CGTGCAGAGTGGAGGGAGAAC-3' and 5'-GCATCTGACTTTGAGCCTCAGG-3'. PCR cycling conditions were: 94°C 5min followed by 94°C 30s, 60°C 30s, 55°C 30s for 40 cycles.

Fluorescent allele-specific oligonucleotide-polymerase chain reaction (ASO-PCR) assay

A fluorescent ASO-PCR was carried out as previously described[1] with modifications. Purified T315I-positive RNA from T315I BCR-ABL cells was diluted with T315I-negative RNA to 1:10, 1:100 and 1:1000, and all RNAs were reverse transcribed using random hexamers and SuperScript III kit (Invitrogen). PCR was carried out with 1 μl of RT reaction each with optimal conditions of 45 cycles and 60^{o}C annealing temperature. The primers for ASO-PCR are as follows: forward outer primer 5'-CGTGAAGACCTTGAAGGAGGACACCATG-3', reverse outer primer 5'-FAM- TTCTCCAGGTACTCCATGGCTGACGAGA-3', reverse T315I mutation primer 5'-FAM- TCCAGGAGGTTCCCGTAGGTCATGAACTAAA-3', forward primer 5'-CCCGGGAGCCCCCGTTCTATATCATAAC -3'. PCR products were diluted and run on ABI 3100 Genetic Analyzer (Applied BioSystems). Care was taken to avoid contamination by performing PCR reactions in a separate room from that for nucleic acid extraction and PCR product electrophoresis, and personal return to PCR reaction room in the same day is prohibited after he handles nuclei acid extraction and electrophoresis.

Bidirectional Pyrophosphorolysis-Activated Polymerization Allele-Specific Amplification (Bi-PAP)

Bi-PAP assay was performed as previously described[2] with the primer pairs (T315I-F*: 5'-GGAGCCCCCGTTCTATATCATCAddT-3') and T315I-R*: 5'-AGGTTCCCGTAGGT-CATGAACTCAddA-3') and PAPase (all purchased from BioVision USA). The PCR cycling conditions were: 1 cycle of 95°C 60s, 60°C 30s, 62°C 30s, 68°C 45s and 72°C 45s followed by 40 or 50 cycles of 95°C 30s, 60°C 30s, 62°C 30s, 68°C 40s and 72°C 5min. Similar to ASO-PCR, Bi-PAP reactions were performed in a separate room from that for nucleic acid extraction and PCR product analysis.

References

1. Willis SG, Lange T, Demehri S, et al. High-sensitivity detection of BCR-ABL kinase domain mutations in imatinib-naive patients: correlation with clonal cytogenetic evolution but not response to therapy. Blood. 2005;106:2128-2137.
2. Shi J, Liu Q, Sommer SS. Detection of ultrarare somatic mutation in the human TP53 gene by bidirectional pyrophosphorolysis-activated polymerization allele-specific amplification. Hum Mutat. 2007;28:131-136.

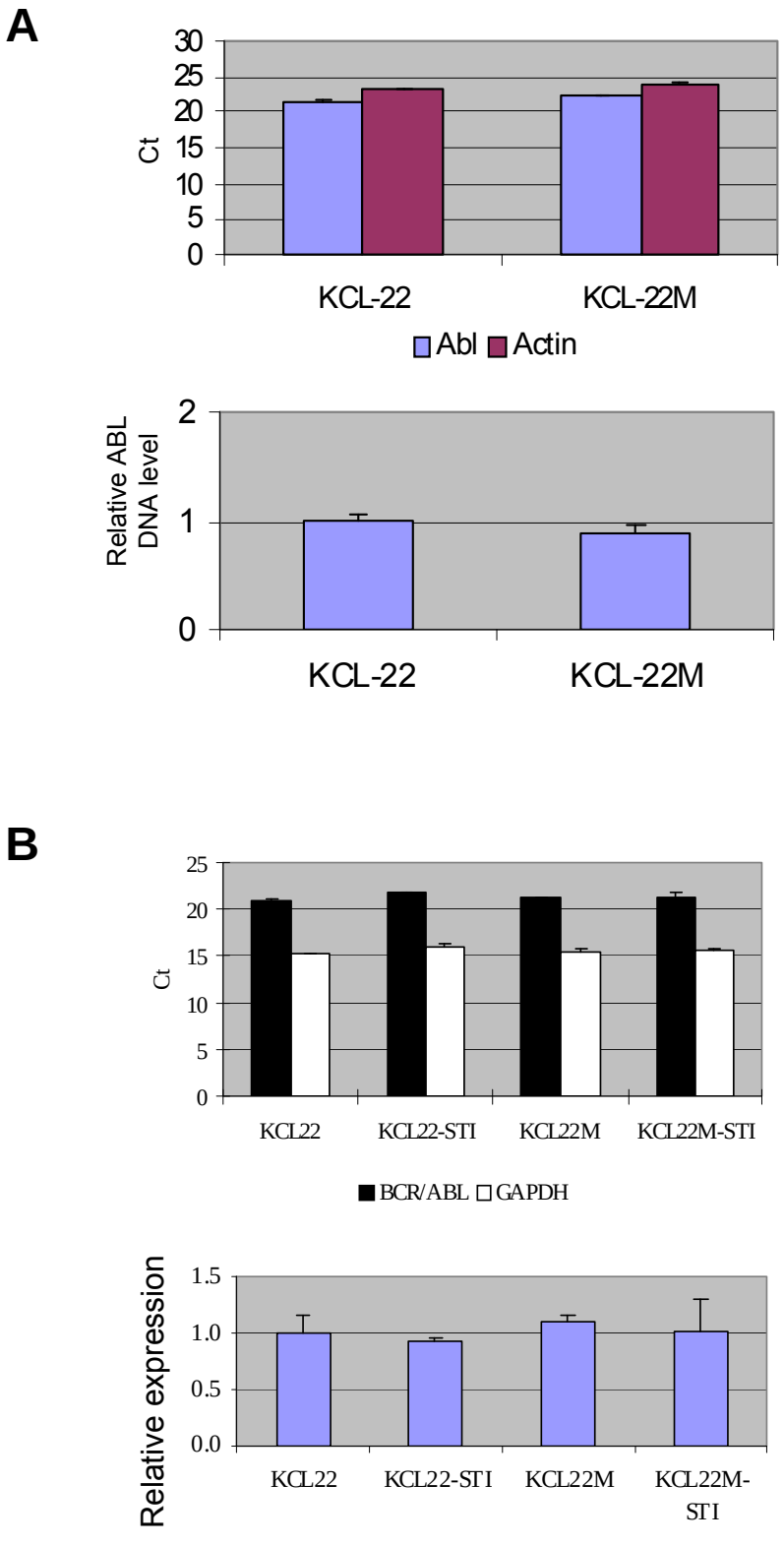
SUPPLEMENTAL FIGURE LEGENDS:

Supplemental Figure 1 Real-time PCR analysis of ABL DNA content and BCR-ABL RNA level . *A,* ABL DNA content in KCL-22M cells was the same as in KCL-22 cells. *B,* BCR-ABL RNA level in KCL-22M cells with or without imatinib (STI) treatment was the same as in KCL-22 cells.

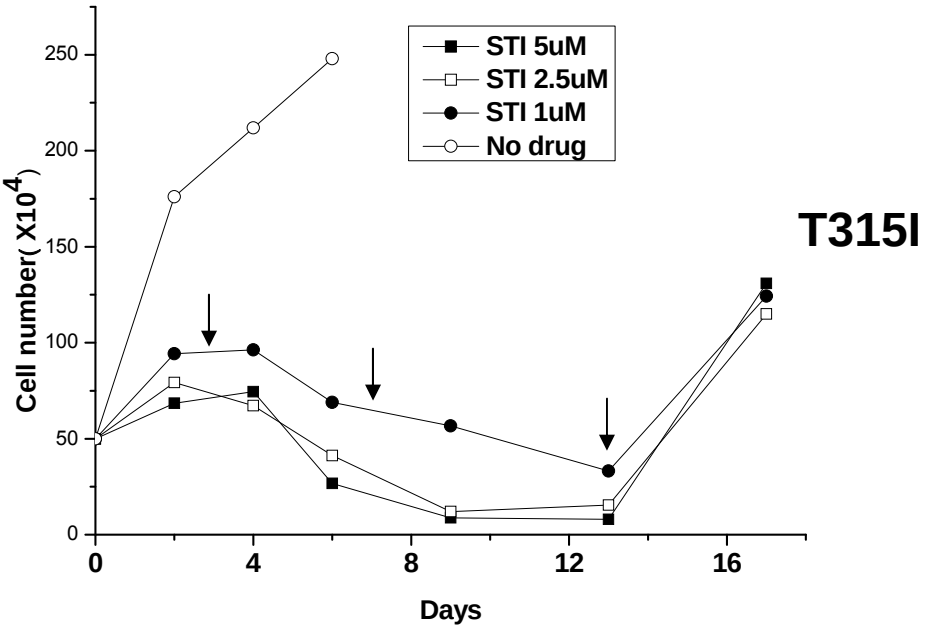
Supplemental Figure 2 Relapse time course of KCL-22 cells on repeated imatinib treatment. Following initial imatinib treatment, fresh imatinib was added at day 3, 7 and 11 (indicated by arrows) by replacing the top half culture medium with the fresh medium. KCL-22 cells relapsed in similar time courses as in the treatment with a single dose of imatinib and developed T315I mutation as well.

Supplemental Figure 3 Detection of T315I mutation by ASO-PCR and Bi-PAP assays *A,* ASO-PCR assay. RNA from KCL-22M cells was serially diluted with RNA from a human osteosarcoma cell line U2OS. ASO-PCR was able to detect at least 1% mutant allele among the wild type allele. No T315I mutation was found in KCL-22 or K562 cells. A pair of outer primers were included in each PCR reaction to amplify a longer fragment of templates as PCR amplification control. *B,* Bi-PAP assay. Various amounts of genomic DNA of KCL-22M cells were mixed with 330ng of U2OS genomic DNA, and Bi-PAP was able to detect 1% T315I mutant allele DNA. No T315I mutation in KCL-22 and clone Ag11 was found with this level of sensitivity.

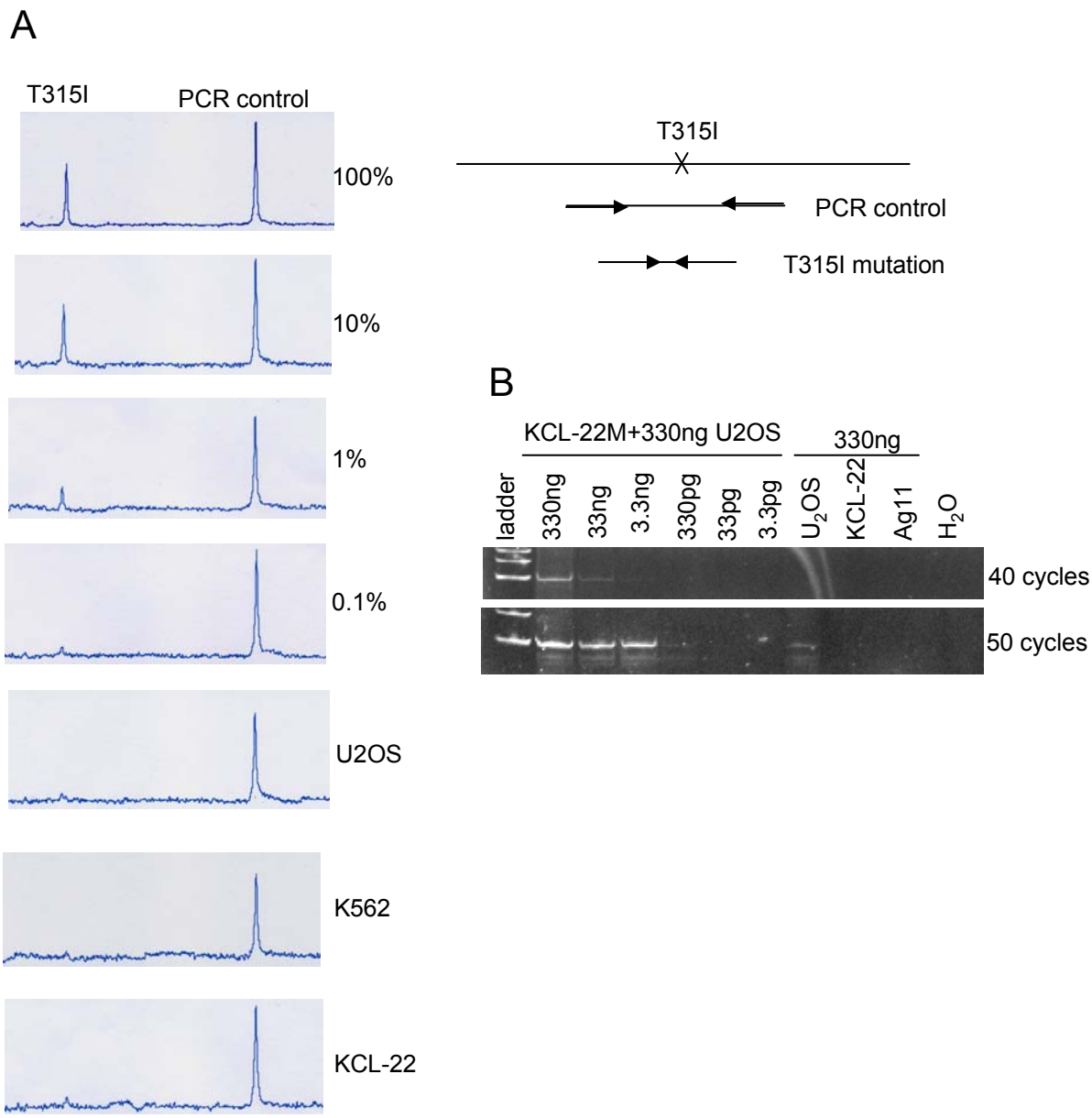
Supplemental Figure 4 Comparison of cell growth of BCR-ABL over-expressing cells. Growth curves (A) and Soft agar plating efficiency (B) of R1, Wt and KI mutant BCR-ABL over-expressing cells as described in Figure 8.



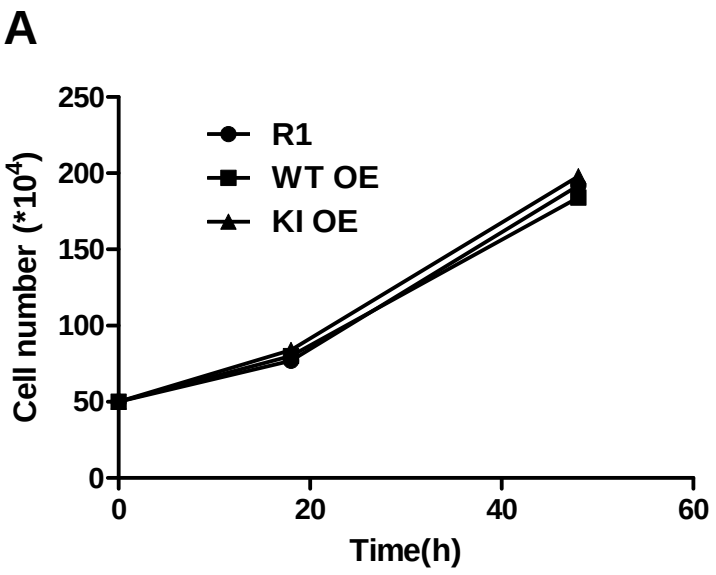
Supplemental Figure 1

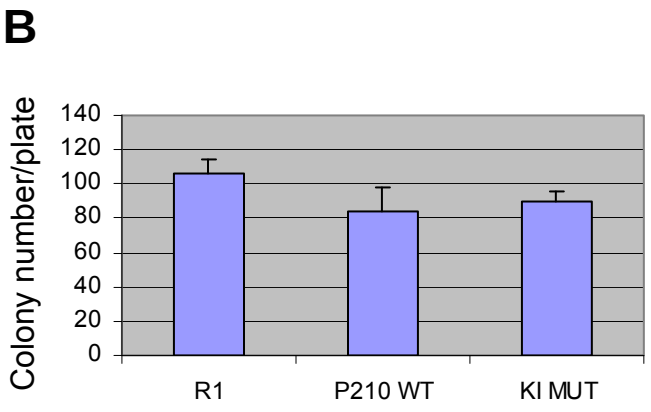


Supplemental Figure 2



Supplemental Figure 3





Supplemental Figure 4