SUPPLEMENTARY APPENDIX

# Ultra-deep sequencing leads to earlier and more sensitive detection of the tyrosine kinase inhibitor resistance mutation T315I in chronic myeloid leukemia

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## Supplementary Materials and Methods

# Ultra-deep sequencing leads to earlier and more sensitive detection of the tyrosine kinase inhibitor resistance mutation T315I in chronic myeloid leukemia

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# Supplemental Tables

## Supplemental Table 1: UDS results

| UPI | Gender Age* | Sample date | Therapy | *BCR-ABL1/ ABL1* (%IS) | T315I (%) | Additional TKI resistance mutations, mutation load (%) | | | | | | | Phase[1] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | f 58,8 | ID: Dec-05, therapy: im | | | | | | | | | | | |
| | | Sep-06 | im | 60.351 | **0** | F359C; 0 | | | | | | | BP |
| | | Oct-06 | ni | 62.838 | **1** | F359C; 0 | | | | | | | BP |
| | | Nov-06 | ni | 85.153 | **84** | F359C; 8 | | | | | | | BP |
| 2 | f 84,4 | ID: Jul-01; therapy: HU, im | | | | | | | | | | | |
| | | Jan-07 | HU | 98.645 | **0** | G250E; 0 | E255K; 0 | E255V; 0 | F359V; 0 | Y253F; 0 | M351T; 0 | H396R; 0 | BP |
| | | Jun-07 | HU | 34.445 | **1** | G250E; 26 | E255K; 6 | E255V; 2 | F359V; 2 | Y253F; 14 | M351T; 11 | H396R; 24 | BP |
| | | Aug-08 | da | 32.870 | **99** | G250E; 0 | E255K; 0 | E255V; 0 | F359V; 0 | Y253F; 0 | M351T; 0 | H396R; 0 | N.A. |
| 3 | m 67,6 | ID: Jul-00; therapy: HU, im | | | | | | | | | | | |
| | | Oct-07 | im | 50.194 | **0** | | | | | | | | N.A. |
| | | Aug-08 | im | 21.501 | **1** | | | | | | | | N.A. |
| | | Dec-08 | da | 33.486 | **9** | | | | | | | | N.A. |
| | | Feb-09 | da | 19.626 | **27** | | | | | | | | N.A. |
| 4 | m 68,9 | ID: Jul-04; therapy: HU, im | | | | | | | | | | | |
| | | Aug-07 | im | 62.013 | **0** | M244V; 5 | F359V; 0 | | | | | | CHR |
| | | Jan-08 | im | 34.743 | **1** | M244V; 95 | F359V; 2 | | | | | | CP |
| | | Apr-08 | im | 47.587 | **11** | M244V; 86 | F359V; 2 | | | | | | CHR |
| | | Nov-08 | im | 33.507 | **40** | M244V; 51 | F359V; 4 | | | | | | CP |
| 5 | m 59,6 | ID: Feb-09; therapy: FLAG-Ida, im, da no MMR | | | | | | | | | | | |
| | | Aug-11 | da | 5.533 | **0** | G250E; 3 | F317L; 25 | F317L; 17 | M244V; 14 | F317L; 12 | F359I; 2 | | N.A. |
| | | Sep-11 | da | 1.222 | **1** | G250E; 3 | F317L; 27 | F317L; 12 | M244V; 8 | F317L; 12 | F359I; 2 | | N.A. |
| | | Oct-11 | da | 3.359 | **1** | G250E; 0 | F317L; 35 | F317L; 16 | M244V; 6 | F317L; 20 | F359I; 0 | | N.A. |
| | | Jan-12 | da | 7.099 | **87** | G250E; 0 | F317L; 7 | F317L; 0 | M244V; 0 | F317L; 1 | F359I; 0 | | N.A. |
| 6 | m 18,8 | ID: Jun-11; therapy: im no MMR | | | | | | | | | | | |
| | | Nov-11 | im | 4.696 | **0** | | | | | | | | N.A. |
| | | Feb-12 | im | 55.383 | **1** | | | | | | | | AP |
| | | May-12 | im | 51.966 | **99** | | | | | | | | N.A. |
| 7 | m 33,5 | ID: Jul-12; therapy: im; da no MMR | | | | | | | | | | | |
| | | Apr-13 | da | 41.933 | **0** | M244V; 0 | | | | | | | N.A. |
| | | Apr-13 | da | 10.960 | **1** | M244V; 0 | | | | | | | CHR |
| | | Jun-13 | da | 16.480 | **4** | M244V; 0 | | | | | | | N.A. |
| | | Jul-13 | da | 6.423 | **16** | M244V; 2 | | | | | | | N.A. |

| | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 8 | f 23,3 | ID: Feb-04; therapy: alloSCT, im | | | | | | | | | | | |
| | | Jan-11 | da | 12.698 | **0** | E255K; 0 | | | | | | | BP |
| | | Mar-11 | im | 3.858 | **2** | E255K; 23 | | | | | | | N.A. |
| | | Jun-11 | da | 38.019 | **57** | E255K; 33 | | | | | | | N.A. |
| 9 | f 56,1 | ID: Nov-06; therapy: im | | | | | | | | | | | |
| | | Dec-07 | da | 27.037 | **2** | F317L; 0 | M244V; 2 | | | | | | BP |
| | | Feb-08 | im | 4.374 | **82** | F317L; 7 | M244V; 4 | | | | | | CP |
| 10 | m 57,2 | ID: Feb-12; therapy: da no MMR | | | | | | | | | | | |
| | | May-12 | da | 15.529 | **0** | | | | | | | | CP |
| | | Aug-12 | da | 2.168 | **3** | | | | | | | | N.A. |
| | | Jan-13 | da | 0.700 | **30** | | | | | | | | N.A. |
| 11 | m 45,6 | ID: Feb-07; therapy: HU, im | | | | | | | | | | | |
| | | Jul-07 | im | 2.231 | **4** | E459K; 1 | | | | | | | N.A. |
| | | Oct-07 | im | 19.529 | **62** | E459K; 7 | | | | | | | CHR |
| 12 | f 72,7 | Mar-10 | ID | 5.378 | **0** | M244V; 0 | | | | | | | BP |
| | | Sep-10 | im | 1.968 | **4** | M244V; 0 | | | | | | | CHR |
| | | Nov-10 | im | 6.986 | **54** | M244V; 10 | | | | | | | N.A. |
| 13 | f 44,8 | ID: Sep-07; therapy: im, HU | | | | | | | | | | | |
| | | May-11 | da | 8.522 | **0** | M244V; 0 | F359V; 84 | E355G; 7 | | | | | N.A. |
| | | May-11 | da | 2.239 | **4** | M244V; 5 | F359V; 67 | E355G; 3 | | | | | N.A. |
| | | Feb-12 | da | 0.610 | **100** | M244V; 0 | F359V; 0 | E355G; 0 | | | | | N.A. |
| 14 | f 63,9 | ID: before Nov-04, therapy: im | | | | | | | | | | | |
| | | Jul-06 | im | 61.908 | **0** | E255K; 0 | M244V; 1 | | | | | | N.A. |
| | | Aug-06 | im | 36.130 | **5** | E255K; 0 | M244V; 0 | | | | | | N.A. |
| | | Apr-07 | im | 68.947 | **34** | E255K; 5 | M244V; 3 | | | | | | N.A. |
| 15 | m 69,9 | ID: Dec-07; therapy: im **CCyR: Apr-08** | | | | | | | | | | | |
| | | Apr-08 | im | 0.118 | **0** | E255V; 0 | Y253H; 0 | L248V; 0 | | | | | AP |
| | | Jul-08 | im | 9.969 | **5** | E255V; 3 | Y253H; 41 | L248V; 22 | | | | | AP |
| | | Nov-08 | da | 66.324 | **99** | E255V; 0 | Y253H; 0 | L248V; 0 | | | | | BP |
| 16 | m 61,3 | Feb-12 | ID | 62.688 | **0** | E255K; 0 | E255V; 0 | F359C; 0 | E355G; 0 | | | | CP |
| | | May-12 | im | 50.435 | **7** | E255K; 63 | E255V; 2 | F359C; 10 | E355G; 8 | | | | BP |
| | | Jul-12 | da | 44.527 | **89** | E255K; 7 | E255V; 0 | F359C; 0 | E355G; 0 | | | | BP |
| 17 | m 69,8 | ID: Nov-00; therapy: HU, im | | | | | | | | | | | |
| | | Jun-06 | im | 37.327 | **8** | G250E; 49 | F359C; 8 | | | | | | CP |
| | | Oct-06 | im | 58.829 | **9** | G250E; 71 | F359C; 12 | | | | | | CP |
| | | Jan-07 | im | 58.751 | **9** | G250E; 76 | F359C; 5 | | | | | | CP |
| | | Feb-08 | im | 40.385 | **4** | G250E; 94 | F359C; 0 | | | | | | CP |
| | | Sep-08 | im | 30.392 | **99** | G250E; 0 | F359C; 0 | | | | | | CP |

| | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 18 | f 52,1 | ID: therapy: Feb-02; HU, IFN, im, da | | | | | | | | | | | |
| | | Jul-07 | da | 17.407 | **0** | | | | | | | | N.A. |
| | | Feb-08 | da | 6.456 | **10** | | | | | | | | N.A. |
| | | May-08 | da | 7.972 | **4** | | | | | | | | N.A. |
| | | Jun-08 | da | 30.130 | **94** | | | | | | | | N.A. |
| 19 | f 62,0 | ID: May-09; therapy: im, ni | | | | | | | | | | | |
| | | Feb-13 | alloSCT | 13.318 | **0** | G250E; 0 | E255K; 0 | E255V; 0 | F317L; 100 | F486S; 0 | | | N.A. |
| | | May-13 | alloSCT | 2.380 | **11** | G250E; 1 | E255K; 9 | E255V; 7 | F317L; 88 | F486S; 2 | | | N.A. |
| | | May-13 | alloSCT | 33.063 | **51** | G250E; 7 | E255K; 6 | E255V; 30 | F317L; 53 | F486S; 7 | | | N.A. |
| 20 | m 61,0 | Sep-11 | ID | 45.975 | **0** | | | | | | | | CP |
| | | Jan-12 | im | 27.036 | **14** | | | | | | | | CP |
| | | Apr-12 | im | 49.833 | **91** | | | | | | | | CP |
| 21 | m 55,7 | ID: Apr-08; therapy: im no MMR | | | | | | | | | | | |
| | | Aug-08 | im | 7.863 | **0** | E255V; 0 | | | | | | | N.A. |
| | | Apr-09 | im | 74.007 | **27** | E255V; 2 | | | | | | | AP |
| 22 | m 42,4 | ID: Nov-01; therapy: HU, alloSCT, IFN | | | | | | | | | | | |
| | | Feb-10 | im | 53.805 | **0** | | | | | | | | BP |
| | | Jun-10 | da | 65.635 | **30** | | | | | | | | N.A. |
| 23 | m 61,4 | ID: Sep-12; therapy: im; da no MMR | | | | | | | | | | | |
| | | Feb-14 | da | 15.362 | **0** | | | | | | | | N.A. |
| | | May-14 | da | 18.336 | **30** | | | | | | | | N.A. |
| 24 | m 81,3 | Nov-11 | ID | 72.252 | **0** | E255K; 0 | E255V; 0 | E459K; 0 | Y253F; 0 | | | | BP |
| | | Jan-12 | im | 52.970 | **31** | E255K; 8 | E255V; 2 | E459K; 15 | Y253F; 3 | | | | BP |
| 25 | m 69,3 | ID: Jun-11, therapy: im | | | | | | | | | | | |
| | | Jan-14 | ni | 26.360 | **0** | F359V; 64 | Y253H; 3 | E450K; 6 | | | | | N.A. |
| | | Aug-14 | da | 38.323 | **40** | F359V; 0 | Y253H; 0 | E450K; 0 | | | | | CP |
| 26 | f 61,4 | ID: Jul-96; therapy: im | | | | | | | | | | | |
| | | Jul-09 | im | 42.570 | **0** | E459K; 3 | | | | | | | N.A. |
| | | Nov-09 | da | 45.103 | **46** | E459K; 0 | | | | | | | BP |
| 27 | m 65,2 | ID: Jul-06; therapy: im | | | | | | | | | | | |
| | | Mar-12 | ni | 2.794 | **0** | | | | | | | | N.A. |
| | | Jun-12 | ni | 2.834 | **46** | | | | | | | | N.A. |
| 28 | m 41,1 | ID: Feb-12; therapy: ni no MMR | | | | | | | | | | | |
| | | May-12 | ni | 7.444 | **0** | | | | | | | | N.A. |
| | | Aug-12 | ni | 66.459 | **49** | | | | | | | | BP |

| | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 29 | m 58,6 | ID: Jan-08; therapy: im no MMR | | | | | | | | | | | |
| | | Sep-08 | im | 43.194 | **0** | F359V; 20 | Y253H; 2 | | | | | | BP |
| | | Dec-08 | da | 48.440 | **55** | F359V; 0 | Y253H; 0 | | | | | | N.A. |
| 30 | m 52,1 | ID: Oct-12; therapy: HU, ni no MMR | | | | | | | | | | | |
| | | Mar-13 | ni | 43.170 | **0** | E255K; 4 | E255V; 3 | F359V; 79 | | | | | N.A. |
| | | May-13 | da | 28.060 | **56** | E255K; 9 | E255V; 0 | F359V; 30 | | | | | BP |
| 31 | m 71,6 | ID: Dec-08; therapy: da, im, cytarabin, HU no MMR | | | | | | | | | | | |
| | | Nov-13 | ni | 39.294 | **0** | F359V; 0 | E255K; 0 | | | | | | CP |
| | | Jan-14 | ni | 47.174 | **64** | F359V; 20 | E255K; 13 | | | | | | CHR |
| 32 | m 59,5 | Dec-08 | ID | 51.109 | **0** | M244V; 0 | | | | | | | CP |
| | | Aug-09 | im | 58.749 | **68** | M244V; 29 | | | | | | | CHR |
| 33 | m 45,8 | ID: Dec-10; therapy: im no MMR | | | | | | | | | | | |
| | | Nov-11 | im | 59.422 | **0** | F317I; 0 | F317L; 0 | Y253H;100 | | | | | BP |
| | | Feb-12 | im | 29.683 | **76** | F317I; 15 | F317L; 7 | Y253H;100 | | | | | BP |
| 34 | m 85,3 | ID: Aug-98; therapy: HU; im, da, ni | | | | | | | | | | | |
| | | Jan-12 | ni | 46.585 | **0** | E255K; 2 | F317L; 0 | V299L; 18 | | | | | CP |
| | | Mar-13 | ni | 53.703 | **88** | E255K; 1 | F317L; 7 | V299L; 0 | | | | | CP |
| 35 | m 62,3 | ID: Sep-10; therapy: da no MMR | | | | | | | | | | | |
| | | Apr-11 | da | 33.591 | **0** | | | | | | | | BP |
| | | Jun-11 | da | 30.393 | **94** | | | | | | | | BP |
| 36 | m 51,1 | ID: Apr-13, therapy: im no MMR | | | | | | | | | | | |
| | | Sep-13 | im | 37.657 | **0** | Q252H; 10 | | | | | | | BP |
| | | Oct-13 | da | 40.200 | **96** | Q252H; 2 | | | | | | | BP |
| 37 | f 61,3 | ID: Jul-06; therapy: im **MMR (Jan-07) CCyR (Mar-08)** | | | | | | | | | | | |
| | | Nov-07 | im | 47.754 | **0** | E255V; 99 | | | | | | | BP |
| | | Apr-08 | da | 5.379 | **100** | E255V; 99 | | | | | | | CP |
| 38 | f 38,8 | ID: Sept-07; therapy cytarabine -daunorubicin, im, alloSCT, da | | | | | | | | | | | |
| | | Oct-08 | ni | 28.947 | **0** | | | | | | | | CHR |
| | | Dec-08 | ni | 0.046 | **100** | | | | | | | | BP |
| 39 | f 60,5 | ID: Oct-01; therapy: im | | | | | | | | | | | |
| | | Mar-09 | im | N.A. | **0** | | | | | | | | BP |
| | | Aug-09 | da | 58.644 | **100** | | | | | | | | BP |

| | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 40 | m<br>72,0 | ID: Apr-10, therapy: im<br>no MMR | | | | | | | | | | | |
| | | May-10 | im | 50.260 | **0** | | | | | | | | BP |
| | | Jul-10 | im | 28.353 | **100** | | | | | | | | BP |

**Supplemental Table 1:** For patients of cohort 1, given are sampling dates, patients' characteristic (gender, age, date of initial diagnosis, disease phase), mutation loads and *BCR-ABL1*/*ABL1* (%IS). In case molecular and/or cytogenetic response data was available since initial diagnosis, achievement or absence of treatment milestones is given.

Abbreviations: UPI indicates unique patient identifier; TKI, tyrosine kinase domain; IS, international scale; N.A., not analyzed; f, female; m, male; ID: initial diagnosis; im, imatinib; da, dasatinib; ni, nilotinib; HU, Hydroxyurea, IFN; Interferon alpha; alloSCT, allogeneic stem cell transplantation, IDA-FLAG, idarubicin, fludarabine, cytarabine, G-CSF; CHR, complete hematologic response; CP, chronic phase; AP, accelerated phase; BP, blast phase (definitions according to World Health Organization1); MMR, major molecular response; CCyR: complete cytogenetic response.
*Age at the time point when T315I had reached the Sanger Sequencing detection limit (end of monitoring period)

**Supplemental Table 2: Cytogenetic and molecular cytogenetic results**

| UPI | Sample date | Additional cytogenetic aberrations affecting the *BCR-ABL1* sequence[1]; ISCN formula | Results FISH |
|---|---|---|---|
| 1 | Sep-06 | no | *BCR-ABL*+ |
| 1 | Oct-06 | N.A. | *BCR-ABL*+ |
| 1 | Nov-06 | no | *BCR-ABL*+ |
| 2 | Jan-07 | yes;<br>47,XX,t(9;22)(q34;q11),+der(22)t(9;22)(q34;q11) | *BCR-ABL*+;<br>duplication of colocalization signal[2] |
| 2 | Jun-07 | No | *BCR-ABL*+ |
| 2 | Aug-08 | N.A. | N.A. |
| 3 | Oct-07 | N.A. | *BCR-ABL*+ |
| 3 | Aug-08 | N.A. | N.A. |
| 3 | Dec-08 | N.A. | N.A. |
| 3 | Feb-09 | N.A. | N.A. |
| 4 | Aug-07 | no | *BCR-ABL*+ |
| 4 | Jan-08 | no | *BCR-ABL*+ |
| 4 | Apr-08 | no | *BCR-ABL*+ |
| 4 | Nov-08 | no | *BCR-ABL*+ |
| 5 | Aug-11 | N.A. | N.A. |
| 5 | Sep-11 | N.A. | N.A. |
| 5 | Oct-11 | N.A. | N.A. |
| 5 | Jan-12 | N.A. | N.A. |
| 6 | Nov-11 | N.A. | N.A. |
| 6 | Feb-12 | yes; 43~44,XY,der(1)t(1;5)(p34;q21)t(1;7)(q21;q31),<br>-3,der(5)t(5;17)(q11;q21),der(6)del(6)(p11)del(6)(q22),-<br>7,t(9;22;12)(q34;q11;q22),<br>der(9)t(6;9)(?q22;p11),der(15)t(3;15)(q13;q25),<br>-17,der(19)t(1;19)(q41;p11), +der(22)t(9;22)(q34;q11) | *BCR-ABL*+;<br>duplication of colocalization signal[2] |
| 6 | May-12 | N.A. | N.A. |
| 7 | Apr-13 | N.A. | N.A. |
| 7 | Apr-13 | no | *BCR-ABL*+ |
| 7 | Jun-13 | N.A. | N.A. |
| 7 | Jul-13 | N.A. | N.A. |
| 8 | Jan-11 | N.A. | N.A. |
| 8 | Mar-11 | N.A. | N.A. |
| 8 | Jun-11 | N.A. | N.A. |
| 9 | Dec-07 | yes; 46,XX,der(5)t(5;7)(q22;q22),<br>-7,t(9;22)(q34;q11),+der(22)t(9;22)(q34;q11) | *BCR-ABL*+;<br>duplication of colocalization signal[2] |
| 9 | Feb-08 | N.A. | *BCR-ABL*+ |
| 10 | May-12 | N.A. | *BCR-ABL*+ |
| 10 | Aug-12 | N.A. | N.A. |
| 10 | Jan-13 | N.A. | N.A. |
| 11 | Jul-07 | N.A. | *BCR-ABL*+ |

| | | | |
|---|---|---|---|
| 11 | Oct-07 | no | *BCR-ABL+* |
| 12 | Mar-10 | no | *BCR-ABL+* |
| 12 | Sep-10 | no | N.A. |
| 12 | Nov-10 | N.A. | N.A. |
| 13 | May-11 | N.A. | N.A. |
| 13 | May-11 | N.A. | N.A. |
| 13 | Feb-12 | N.A. | N.A. |
| 14 | Jul-06 | N.A. | *BCR-ABL+*;<br>duplication of colocalization signal[2] |
| 14 | Aug-06 | N.A. | *BCR-ABL+*;<br>duplication of colocalization signal[2] |
| 14 | Apr-07 | yes;<br>47,XX,t(9;22)(q34;q11), +der(22)t(9;22)(q34;q11) | *BCR-ABL+*;<br>duplication of colocalization signal[2] |
| 15 | Apr-08 | No | BCR-ABL- |
| 15 | Jul-08 | No | *BCR-ABL+* |
| 15 | Nov-08 | N.A. | N.A. |
| 16 | Feb-12 | N.A. | *BCR-ABL+* |
| 16 | May-12 | no | N.A. |
| 16 | Jul-12 | N.A. | N.A. |
| 17 | Jun-06 | no | *BCR-ABL+* |
| 17 | Oct-06 | no | *BCR-ABL+* |
| 17 | Jan-07 | no | *BCR-ABL+* |
| 17 | Feb-08 | no | *BCR-ABL+* |
| 17 | Sep-08 | no | *BCR-ABL+* |
| 18 | Jul-07 | N.A. | N.A. |
| 18 | Feb-08 | N.A. | N.A. |
| 18 | May-08 | N.A. | N.A. |
| 18 | Jun-08 | no | *BCR-ABL+* |
| 19 | Feb-13 | N.A. | N.A. |
| 19 | May-13 | N.A. | N.A. |
| 19 | May-13 | N.A. | N.A. |
| 20 | Sep-11 | no | *BCR-ABL+* |
| 20 | Jan-12 | N.A. | N.A. |
| 20 | Apr-12 | N.A. | N.A. |
| 21 | Aug-08 | N.A. | *BCR-ABL+* |
| 21 | Apr-09 | yes;<br>47,XY,t(9;22)(q34;q11),dup(17)(q23q25), +der(22)t(9;22)(q34;q11) | *BCR-ABL+*;<br>duplication of colocalization signal[2] |
| 22 | Feb-10 | yes;<br>48,XY,+8,t(9;22)(q34;q11), +der(22)t(9;22)(q34;q11) | *BCR-ABL+*;<br>duplication of colocalization signal[2] |
| 22 | Jun-10 | yes;<br>46,XY,t(9;22)(q34;q11), +der(22)t(9;22)(q34;q11) | *BCR-ABL+*;<br>duplication of colocalization signal[2] |
| 23 | Feb-14 | N.A. | N.A. |
| 23 | May-14 | N.A. | N.A. |
| 24 | Nov-11 | no | *BCR-ABL+* |
| 24 | Jan-12 | N.A. | N.A. |

| | | | |
|---|---|---|---|
| 25 | Jan-14 | N.A. | N.A. |
| 25 | Aug-14 | N.A. | N.A. |
| 26 | Jul-09 | N.A. | N.A. |
| 26 | Nov-09 | yes; 47,XX,-7,+8,+8,der(8;12)(p10;q10), t(9;22)(q34;q11),i(17)(q10), +der(22)t(8;22)(q11;q11)t(8;12)(q24;p11) t(12;22)(p13;q11)t(9;22)(q34;q11) | *BCR-ABL*+; duplication of colocalization signal[2] |
| 27 | Mar-12 | N.A. | N.A. |
| 27 | Jun-12 | N.A. | N.A. |
| 28 | May-12 | N.A. | N.A. |
| 28 | Aug-12 | yes; 46,XY,dic(7;9)(p12;p12),t(9;22)(q34;q11), +der(22)t(9;22)(q34;q11) | *BCR-ABL*+; duplication of colocalization signal[2] |
| 29 | Sep-08 | N.A. | N.A. |
| 29 | Dec-08 | no | *BCR-ABL*+; duplication of colocalization signal[2] |
| 30 | Mar-13 | N.A. | *BCR-ABL*+ |
| 30 | May-13 | no | N.A. |
| 31 | Nov-13 | N.A. | N.A. |
| 31 | Jan-14 | N.A. | N.A. |
| 32 | Dec-08 | no | *BCR-ABL*+ |
| 32 | Aug-09 | no | *BCR-ABL*+ |
| 33 | Nov-11 | no | *BCR-ABL*+ |
| 33 | Feb-12 | no | *BCR-ABL*+ |
| 34 | Jan-12 | N.A. | N.A. |
| 34 | Mar-13 | N.A. | N.A. |
| 35 | Apr-11 | yes; 48,XY,t(9;22)(q34;q11),+21, +der(22)t(9;22)(q34;q11) | N.A. |
| 35 | Jun-11 | no | N.A. |
| 36 | Sep-13 | no | *BCR-ABL*+ |
| 36 | Oct-13 | N.A. | N.A. |
| 37 | Nov-07 | no | *BCR-ABL*+ |
| 37 | Apr-08 | no | *BCR-ABL*+ |
| 38 | Oct-08 | no | *BCR-ABL*+ |
| 38 | Dec-08 | no | *BCR-ABL*- |
| 39 | Mar-09 | no | *BCR-ABL*+ |
| 39 | Aug-09 | N.A. | N.A. |
| 40 | May-10 | no | *BCR-ABL*+ |
| 40 | Jul-10 | N.A. | *BCR-ABL*+ |

**Supplemental Table 2:** For patients of cohort 1, given are additional cytogenetic aberrations.

Abbreviations: N.A. indicates not analyzed; FISH, Fluorescence in situ hybridization; ISCN: International System of Cytogenetic Nomenclature
[1]Listed are only cytogenetic aberrations in addition to the Philadelphia chromosome affecting the *BCR-ABL1* sequence. Patients with other chromosomal aberrations are listed in the category "no". For 14/42 patients of cohort 2 cytogenetic or molecular cytogenetic data was available. No aberration affecting the *BCR-ABL1* copy number was detected.
[2]duplication of colocalization signal indicates duplication of Philadelphia chromosome

**Supplemental Table 3: Primer sequences**

| | |
|---|---|
| 454_1st PCR-F | GAGCAGCAGAAGAAGTGTTTCAGA |
| 454_1st PCR-R | CTTGGAGTGAGGCATCTCAG |
| 454_Seq1-F | TGTCTATGGTGTGTCCCCCA |
| 454_Seq2-F | TGCTGTACATGGCCACTCAG |
| 454_Seq1-R | ACTTGTTGTAGGCCAGGCTC |
| 454_Seq2-R | CCTGCAGCAAGGTACTCACA |

**Supplemental Table 3:** Primers given in table were used to generate amplicons for 454 XL+ sequencing.

**Supplemental Table 4: PCR amplification**

| | | |
|---|---|---|
| ***BCR-ABL1* fusion transcript amplification (1st PCR):** | | |
| 95 °C | 5 min | |
| 95 °C | 30 sec | x29 |
| 60 °C | 30 sec | |
| 72 °C | 3 min 30 sec | |
| 72 °C | 7 min | |
| | | |
| **Sequencing amplicon amplification (nested PCR)** | | |
| 95 °C | 5 min | |
| 95 °C | 30 sec | x29 |
| 60 °C | 30 sec | |
| 72 °C | 50 sec | |
| 72 °C | 7 min | |

**Supplemental Table 4:** PCR conditions to generate amplicons for 454 XL+ sequencing are given.

**Supplemental Table 5: Dilution experiment**

| | | mutation load | | | | |
|---|---|---|---|---|---|---|
| | mixing ratio | **Q252H** | **E255K** | **T315I** | **F359V** | **E499E** |
| Sample A | 90 | 2% | | 97% | | |
| Sample B | 10 | | 14% | 64% | 21% | 100% |
| **calculated mutation load** | | **2%** | **1%** | **93%** | **2%** | **10%** |

| | mixing ratio | **Q252H** | **E255K** | **T315I** | **F359V** | **E499E** |
|---|---|---|---|---|---|---|
| Sample A | 99 | 2% | | 97% | | |
| Sample B | 1 | | 14% | 64% | 21% | 100% |
| **calculated mutation load** | | **2%** | **0%*** | **97%** | **0%*** | **1%** |

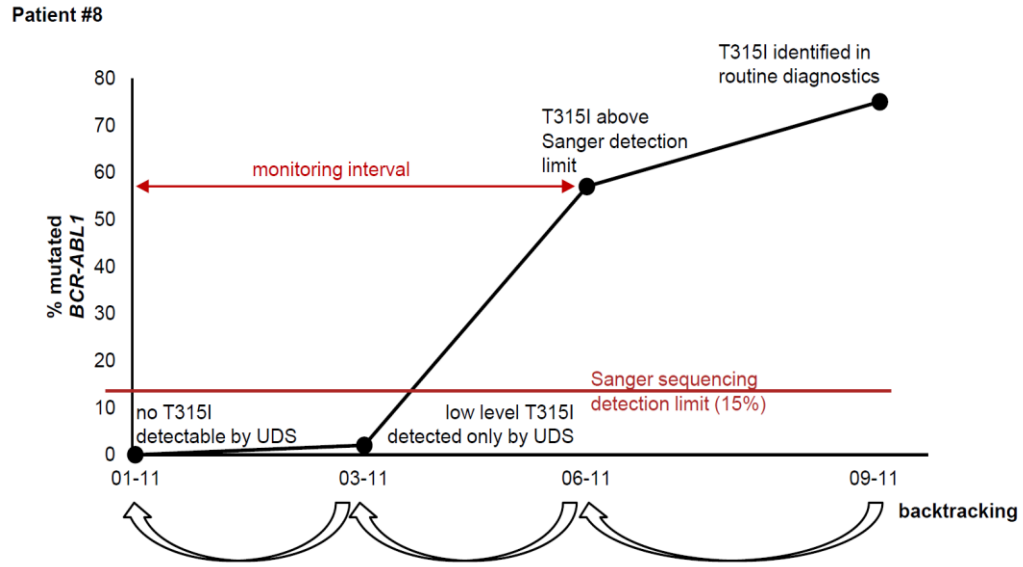
| | mixing ratio | **M244V** | **E255K** | **T315I** |
|---|---|---|---|---|
| Sample C | 1 | 2% | | 61% |
| Sample D | 2 | | 99% | |
| **calculated mutation load** | | **1%** | **66%** | **20%** |

**Supplemental Table 5:** Samples were mixed in the indicated ratio to simulate mutations with low and defined loads.

*Mutation loads below 1% are not called.

## Supplemental Figures

### Figure 1




**Supplemental Figure 1:** Patient #8 illustrates our study design. From the initial time point when T315I was identified during routine diagnostics, we backtracked earlier samples until no T315I mutation was detectable. We selected the sample from June 2011 as endpoint of the monitoring interval, because the T315I mutation load had reached the Sanger sequencing detection limit, and excluded the sample from September 2011.

## Figure 2


**Supplemental Figure 2:** Individual sequencing reads (33 of 2562) of patient #33 (s. Figure 5D) are shown. The c.944C>T (T315I), the c.949T>A (F317I) and the c.951T>A (F317L) mutated bases are highlighted in yellow.

## References

1. Baccarani M, Deininger MW, Rosti G, et al. European LeukemiaNet recommendations for the management of chronic myeloid leukemia: 2013. Blood. 2013;122(6):872-884.