

## 517 Spirit 2: An NCRI Randomised Study Comparing Dasatinib with Imatinib in Patients with Newly Diagnosed CML

<https://ash.confex.com/ash/2014/webprogram/Paper66809.html>

**Objective.** SPIRIT 2 is the largest phase 3 prospective randomized open-label trial comparing imatinib 400mg with dasatinib 100mg daily: this is the first presentation of data comparing the two arms.

**Methods.** 814 patients were recruited at 144 hospitals between August 2008 and March 2013. 812 started study medication (406 in each arm). The primary endpoint is event-free survival at 5 years. A key secondary endpoint is the rate of achievement of a BCR-ABL/ABL ratio of  $<0.1\%^{15}$  (major molecular response (MMR), 3 log reduction or  $MR^3$ ).

### Results.

**Discontinuations.** With a median follow up of 34 months a total of 289/812 (35.6%) patients have discontinued study medication. 118/812 (14.5%) patients have discontinued due to non-haematological toxicity: imatinib 47/406 (11.6%); dasatinib 71/406 (17.5%). 40 patients discontinued due to sub optimal response as assessed by the treating physician: imatinib 37/406 (9.1%); dasatinib 3/406 (0.7%).

**Side effects.** Patients receiving imatinib experienced GI toxicity more often than patients receiving dasatinib; fatigue, rash and headache were more common with dasatinib. A higher rate of grade 3/4 thrombocytopenia was observed in the dasatinib arm: imatinib 17/406 (4.2%); dasatinib 52/406 (12.8%). Pleural effusions occurred in 78/406 (19.2%) patients on dasatinib; 13 of 78 (16.7%) patients required drainage. Arterial cardiovascular events (excluding hypertension) were experienced by 10/812 (1.2%) patients: imatinib 2/406 (0.5%); myocardial infarction (MI) x2; dasatinib 8/406 (2.0%); MI x1; angina/acute coronary syndrome x5; peripheral arterial disease x2). Hypertension was observed in 10/812 (1.2%) patients: imatinib 3/406 (0.7%); dasatinib 7/406 (1.7%). Venous CV events occurred in 7/812 (0.9%) patients: imatinib 3/406 (0.7%); dasatinib 4/406 (1.0%).

**Efficacy.** For both PCR and cytogenetic analyses patients that had discontinued their allocated therapy or that did not have a 12 month sample were analysed as not having achieved  $MR^3$ /CCR. The  $MR^3$  (PCR  $<0.1\%^{15}$ ) rate at 12 months in all treated patients is significantly different ( $p<0.001$ ) between the two treatment arms: imatinib 173/406 (42.6%); dasatinib 236/406 (58.1%). The  $MR^3$  rate at 12 months in patients treated with dasatinib is 51/78 (65.4%) in those with a pleural effusion and 185/328 (56.4%) in those without ( $p=0.148$ , NS). The complete cytogenetic response (CCR) rate at 12 months is: imatinib 163/406 (40.1%); dasatinib 207/406 (51.0%). The difference between the two treatment arms is statistically significant ( $p=0.002$ ) but caution is required in interpreting these data as there were missing analyses in 367 of 812 (45.2%) patients: imatinib 191 of 406 (47.0%), dasatinib 176 of 406 (43.3%). The difference in major cytogenetic response (MCR) rate between the two treatment arms at 12 months is not statistically significant: imatinib 200/406 (49.3%); dasatinib 218/406 (53.7%),  $p=0.206$ .

**Disease progression and deaths.** 16 patients have progressed to either accelerated phase or blast crisis and 13 of those progressions were within the first year. Accelerated phase: imatinib 3/406 (0.7%); dasatinib 2/406 (0.5%). Blast crisis: imatinib 7/406 (1.7%); dasatinib 4/406 (1.0%).

**Conclusions.** Dasatinib-treated patients have a higher rate of molecular response at 1 year but, with a median of 34 months follow up, there is no significant difference in rates of disease progression or overall survival. More patients abandoned imatinib than dasatinib due to investigator concerns about sub optimal responses. Further follow up is required to evaluate whether there will be differences in event free survival at five years.

## 151 Interim Analysis of a Pan European Stop Tyrosine Kinase Inhibitor Trial in Chronic Myeloid Leukaemia: The EURO-SKI study

<https://ash.confex.com/ash/2014/webprogram/Paper74060.html>

**Background:** The tyrosine kinase inhibitors (TKIs) have dramatically changed the natural history of chronic myeloid leukaemia (CML) leading to significant improvement in clinical outcome and survival rates. The option of treatment cessation has recently become of utmost importance. Indeed, prospective trials suggest that imatinib therapy may be safely and successfully discontinued in CML pts with deep and sustained molecular responses (Mahon Lancet Oncol 2010, Ross Blood 2013). The major aim of the EURO-SKI study (European Leukaemia Net Stop TKI study) was to define prognostic markers to increase the rate of patients in durable deep MR after stopping TKI. Further aims were the evaluation of harmonized methods of molecular monitoring, assessment of quality of life, and calculation of saved treatment costs per country.

**Methods:** Adult CML patients in chronic phase CML on TKI treatment in confirmed deep molecular response (MR<sup>4</sup>, BCR-ABL <0.01%) for at least one year (>4 log reduction on TKI therapy for >12 months confirmed by three consecutive PCR tests) and under TKI treatment for at least 3 years were eligible. MR<sup>4</sup> confirmation was performed in a standardized laboratory (n=6). Primary endpoint was the assessment of the duration of MR (defined by continuous MMR) after stopping TKI. Patients (pts) after a prior TKI failure were excluded. According to protocol, an interim analysis was planned after 200 patients with eligible molecular results at month (mo) 6 were available to test the null hypothesis that relapse-free survival at 6 mo is less or equal 40%.

**Results:** From June 2012 to June 2014, 498 CML pts in chronic phase from 10 countries were enrolled and included in the trial. From June 2012 to July 2013, 254 pts from 8 countries were registered; 54 were excluded (consent withdrawal n=1, protocol violation n=1, not eligible n=34, restart of TKI without relapse n=4, atypical or unknown transcript n=6, missing data n=8). Of the eligible 200 pts, 41.5% were female. Median age at diagnosis was 53.3 years (range, 13.8 to 85.5). In assessable pts 8.7% and 18.2 % were high-risk according to EUTOS and Sokal Scores. 103 pts were treated prior to the start TKI therapy, mostly with Hydroxyurea or interferon. 1st-line TKI was imatinib in 97%, dasatinib in 1.5%, and nilotinib in 1.5% of pts. Twenty-four pts switched to second-line TKI therapy due to intolerance, 16 to dasatinib, 2 to imatinib, and 6 to nilotinib. The median time from diagnosis of CML to TKI cessation was 8 years (range, 3-19 years). TKI treatment duration was less than 5 years in 16%, 5-8 years in 36% and > 8 years in 48% of pts. Median duration of TKI treatment was 8 years (range, 3-12.6 years) and median duration of MR<sup>4</sup> before TKI cessation was 5.4 years (range, 1-11.7 years). MR<sup>4</sup> duration was less than 2 years in 8%, 2-5 years in 37%, 5-8 years in 39% and >8 years in 16% of pts. For all eligible pts, a standardized European laboratory confirmed MR<sup>4</sup> assessment. Since 123 of the 200 pts (61.5%, 95% CI: [54.4%; 68.3%]) remained without relapse the first 6 mo, the null hypothesis could be discarded (p<0.0001).

Recurrence of CML, defined as loss of MMR, was observed in 43/92 pts (47%) treated <8 years, as compared to 23/87 pts (26%) treated for >8 years (p= 0.005). So far, there was a trend for prognostic significance of MR<sup>4</sup> duration: 33/71 pts with MR<sup>4</sup> <5 years (46%) lost MMR within 6 mo as compared to 28/87 pts (32%) with MR<sup>4</sup> duration >5 years (p=0.07).

No significant difference was observed for relapse within 6 mo according to depth of molecular response at discontinuation (MR<sup>4</sup> vs MR<sup>4.5</sup> vs MR<sup>5</sup>).

TKI cessation was a safe procedure but a substantial proportion of pts reported transitory musculoskeletal pain starting within weeks after imatinib discontinuation. The phenomenon was described in 30% of Swedish patients as a "TKI withdrawal syndrome" (Richter JCO 2014).

Taking into account the cost of imatinib in Europe and time without treatment in the total study population at the most recent analysis, total savings for the community within the EURO-SKI trial were estimated at 7 million Euros.

**Conclusion:** Employing a standardized molecular testing for patient selection within a TKI cessation trial in CML the chance to stay in treatment-free remission could be higher than previously reported. The EURO-SKI trial will further elucidate the prognostic factors but the preliminary results confirm (as reported in the STIM Study) the prognostic impact of the duration of TKI therapy before stopping.

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## 1797 *BCL2L11* (*BIM*) Deletion Polymorphism Is Associated with Molecular Relapse after ABL Tyrosine Kinase Inhibitor Discontinuation in Patients with Chronic Myeloid Leukaemia with Complete Molecular Response

<https://ash.confex.com/ash/2014/webprogram/Paper71104.html>

### Background:

The inhibition of BCR-ABL1 kinase with tyrosine kinase inhibitors (TKIs) has markedly improved the prognosis of chronic myeloid leukaemia (CML). Recently, it has been recognized that some CML patients with a complete molecular response (CMR) are able to maintain treatment-free remission (TFR) after discontinuation of TKIs. However, no predictive prognostic factors for successful discontinuation of the treatment have yet been identified. We set out to further clarify the role of predictive biomarkers in molecular relapse and non-relapse after ABL TKI discontinuation.

### Materials and methods:

Patients in sustained CMR (MR 4.5) undergoing TKI therapy were eligible for inclusion in the study. Molecular relapse was defined as loss of major molecular response (MMR) of at least one point. Genomic DNA was obtained from whole blood using a DNA Extractor WB Kit (Wako, Osaka, Japan), and was subjected to polymerase chain reaction (PCR) amplification using primers designed to detect a deletion site (2903 bp) in intron two of the *BCL2L11* gene (forward: 5'-AATACCACAGAGGCCACAG-3'; reverse: 5'-GCCTGAAGGTGCTGAGAAAG-3') and JumpStart RedAccuTaq LA DNA polymerase (Sigma Aldrich, St. Louis, MO, USA).

### Results:

32 CML patients (17 men, 15 women, median age 58.4 years) were included in this study (Sokal category; low 24, intermediate 7, high 1). Six patients were treated with IFN $\alpha$  before TKI treatment, and 3 were treated with IFN $\alpha$  after stopping TKI. Median duration from TKI initiation to discontinuation was 79.3 months (range; 22 to 138 months); median duration of CMR before TKI discontinuation was 47.3 months (range; 5 to 97 months). Seven patients showed loss of MMR; 6 relapsed within 6 months and one showed late relapse at 25 months after discontinuation. The cumulative incidence of MMR loss was estimated as 18.8% at 12 months and at 24 months. Fluctuation of BCR-ABL transcript levels below the MMR threshold (> two consecutive positive values) was observed in 6.25% of patients at 24 months after ABL TKI discontinuation. Treatment-free remission was estimated as 81.2% at 12 months and at 24 months. The median period of restoration of second CMR was 6.0 months in re-treated patients. No patient died during the follow-up period. TKI-free remission was estimated as 78.1% at 30

months. There was only a significant difference in *BCL2L11* (*BIM*) deletion polymorphism between the patients who maintained and those who lost MMR ( $p = 0.0253$ ). No significant difference was observed in prior IFN $\alpha$  therapy, time to complete cytogenetic response (CCyR), time to MMR, and time to CMR between relapsing and non-relapsing patients.

**Conclusion:**

Our study shows a specific association between *BCL2L11* (*BIM*) deletion polymorphism and clinical outcome after ABL TKI discontinuation in patients with long-lasting molecular undetectable residual disease. *BCL2L11* (*BIM*) deletion polymorphism may predict relapse after ABL TKI discontinuation, which may have an impact on future ABL TKI discontinuation trials. These results further illustrate the importance of single nucleotide polymorphisms in successful long-term treatment of CML.

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## 1799 The Effect of Nilotinib in Chronic Myeloid Leukaemia Treatment Dose on Spermatogenesis and Folliculogenesis in a Healthy Mouse Model

<https://ash.confex.com/ash/2014/webprogram/Paper71346.html>

**Introduction & Aim:** Chronic myeloid leukaemia (CML) is a hematopoietic pluripotent stem cell disease where myeloid cells lead to uncontrolled proliferation. Current treatment of Ph (+) CML is based on the inhibition of tyrosine kinase inhibitors (TKI), especially second generation drugs. Majority of CML patients are male and 46% of them are between 20 and 64 years of age. Therefore, it is conceivable that inhibition of c-kit or PDGFR by TKI may have deleterious effects on spermatogenesis or folliculogenesis, resulting in male or female subfertility. Aim of this study is to determine the effect of nilotinib on spermatogenesis and folliculogenesis which is used routinely to treat CML.

**Material & Method:** Here we present the results of testicular and ovarian changes after nilotinib administration to five-week old male and female C57bl6 mice. Mice received 0.4 mg of nilotinib per day dissolved in the drinking water for 2 months. Control group received only drinking water. Treatment dose was determined according to the clinical studies regarding the plasma concentrations (20 mg/kg, orally). After scarification of both groups, testicular and ovarian tissues were fixed and paraffin sections were stained with hematoxyline-eosin. In the ovaries, the follicles were counted and their developmental stages were recorded from the serial sections. In the testes, 24 seminiferous tubules with approximately circular cross-sectional profiles were assessed using a classification according to the degree of spermatogenic activity to generate a mean score for each mouse. In addition, tubule diameters were measured using an eyepiece micrometre to provide an additional indication of the level of function.

**Results:** There was less distance between the cortex and medulla than normal, and the follicles were widely scattered instead of organized in a normal hierarchy from the least mature at the periphery to the largest growing stages towards the medulla. The numbers of follicles were significantly different between nilotinib and control groups ( $268 \pm 110$  vs.  $170 \pm 60$ ;  $p = 0.03$ ). Virtually every seminiferous tubule from all animals had active spermatogenesis with either spermatids or spermatozoa present. Mean tubular diameter measurements were  $190,61 \pm 8,33$  vs.  $194,32 \pm 7,26$  in control and nilotinib groups, respectively ( $p = 0.475$ ). Spermatogenic activity index were not significantly different between control and nilotinib groups ( $3.1$  vs.  $3.4$ ;  $p = 0.241$ ).

**Conclusion:** Unlike the manufacturer's results; we showed the suppression of folliculogenesis and prevention of spermatogenesis during the long-term nilotinib treatment. Our results

indicate that nilotinib, within the dose of CML treatment regimen, may create gonadotoxicity and therefore its usage may be an indication for fertility preservation. In the second part of our ongoing study, we are investigating the effect of nilotinib on fertility and teratogenicity.

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## 152 Final Study Results of the Phase 3 Dasatinib versus Imatinib in Newly Diagnosed Chronic Myeloid Leukaemia in Chronic Phase (CML-CP) Trial (DASISION, CA180-056)

<https://ash.confex.com/ash/2014/webprogram/Paper68183.html>

**Background:** The randomized, phase 3 DASISION trial demonstrated improved efficacy with dasatinib compared with imatinib in treatment-naïve CML-CP patients (pts). Dasatinib was also well tolerated, and demonstrated a faster response at 3 months. Here, we report the results of the final, 5-year analysis of DASISION.

**Conclusion:** At 5 years, dasatinib 100 mg once daily has demonstrated superior outcome compared to imatinib 400 mg once daily as initial therapy for CML. This is manifested by a faster time to cytogenetic and molecular responses, with more pts achieving BCR-ABL  $\leq 10\%$  at 3 months, sustained higher cumulative rates of response, and a lower rate of transformation. The 5-year rates of PFS and OS were equal in both arms. After 5 years, no new safety signals have been reported. These consistent results suggest that dasatinib offers meaningful advantages for pts with newly diagnosed CML-CP and remains a standard of care in this setting.

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## 519 Epic: A Phase 3 Trial of Ponatinib Compared with Imatinib in Patients with Newly Diagnosed Chronic Myeloid Leukaemia in Chronic Phase (CP-CML)

<https://ash.confex.com/ash/2014/webprogram/Paper70542.html>

**Background:** Ponatinib is an approved potent oral tyrosine kinase inhibitor active against native and mutated forms of BCR-ABL, including T315I. The phase 2 PACE study demonstrated that ponatinib is highly active in heavily pre-treated Philadelphia chromosome-positive leukaemia patients. Ponatinib efficacy and safety were evaluated in newly diagnosed CP-CML patients in the EPIC trial.

**Methods:** EPIC was a multicentre, international, phase 3, randomized, 2-arm, open-label trial of ponatinib (45 mg once daily) compared with imatinib (400 mg once daily) in newly diagnosed CP-CML; patients were stratified by Sokal risk score (low [ $<0.8$ ] vs intermediate [ $0.8$  to  $\leq 1.2$ ] vs high [ $>1.2$ ]). On 18 October 2013, EPIC was terminated due to the observation of arterial thrombotic events in the ponatinib development program. Consequently, none of the prospectively defined endpoints could be analysed. Data as of 1 April 2014 are presented for endpoints that could be analysed: BCR-ABL<sup>IS</sup>  $<10\%$  rate at 3 months; major molecular response (MMR), molecular response (MR)4, and MR4.5 rates at and after at least 3, 6, 9, and 12 months



and at any time; time to response; complete cytogenetic response rates at 6 and 12 months and any time; and safety.

**Results:** At the time of study termination, 307 patients had been randomized; median follow-up was 5.1 (0.03-17.6) months. Groups were well-balanced with respect to sex, age, pre-treatment, and Sokal score; however, the proportion of patients with 1 or more cardiovascular risk factors (hypertension, hypercholesterolaemia, diabetes, obesity and smoking) was higher in the ponatinib arm (n=97, 63%) compared to the imatinib arm (n=79, 52%). Data were available on 306 treated patients (154 ponatinib, 152 imatinib). Fourteen ponatinib and 2 imatinib patients discontinued due to adverse events (AEs). Molecular response rates for ponatinib were uniformly higher compared with imatinib for all response measures and at all time points (**Table**). The percentage of patients who achieved <10% BCR-ABL at 3 months was significantly higher in the ponatinib compared with imatinib arm overall (Table), and when patients were stratified by high-risk, intermediate-risk, and low-risk Sokal score (Figure). The percentage of patients who achieved MMR, MR4, and MR4.5 at any time in all Sokal risk groups was higher for ponatinib than imatinib (**Figure**). The most common ( $\geq 25\%$ ) all-grade treatment-emergent AEs with ponatinib were rash (38%), abdominal pain (36%), headache (33%), constipation (27%), increased lipase (27%), myalgia (26%), and thrombocytopenia (25%); with imatinib, they were nausea (34%), muscle spasms (34%), and diarrhoea (27%). Twelve percent of ponatinib and 7% of imatinib patients had grade 3/4 thrombocytopenia; 3% of ponatinib and 8% of imatinib patients had grade 3/4 neutropenia. Serious treatment-emergent AEs (SAEs) occurring in  $\geq 3$  ponatinib patients were pancreatitis (n=5), atrial fibrillation (n=3), and thrombocytopenia (n=3); no individual SAEs occurred in  $\geq 3$  imatinib patients. Eleven (7%) ponatinib and 3 (2%) imatinib patients experienced arterial thrombotic events, designated serious for 10 [7%] ponatinib and 1 [0.7%] imatinib patient(s). One patient in the ponatinib arm experienced a serious venous thromboembolic event: there were none in the imatinib arm. Ten of 11 ponatinib patients, and 2 of 3 imatinib patients with arterial thrombotic events had 1 or more cardiovascular risk factors.

**Conclusions:** Despite early termination, at a median follow-up of 5 months, preliminary evidence suggests that ponatinib has improved efficacy over imatinib in newly diagnosed CP-CML patients, but has a higher AE rate, including ATEs at the dose studied. Future investigations of ponatinib in the frontline setting will likely use lower doses and account for relevant risk factors.

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## 518 Achieving Early Landmark Response Is Predictive of Outcomes in Heavily Pre-treated Patients with Chronic Phase Chronic Myeloid Leukaemia (CP-CML) Treated with Ponatinib

<https://ash.confex.com/ash/2014/webprogram/Paper72705.html>

**Background:** Ponatinib is an approved potent, oral, pan-BCR-ABL inhibitor active against native and mutant BCR-ABL. Ponatinib had substantial clinical activity in the phase 2 PACE trial in patients (pts) resistant or intolerant to dasatinib or nilotinib or with the T315I mutation. In the frontline setting, positive associations between achieving response at an early time point (landmark) and long-term outcomes have been shown for tyrosine kinase inhibitors (TKIs) in CML pts. Since landmark analyses have not been reported for a heavily pre-treated population,

in particular 3<sup>rd</sup> line and beyond, this retrospective analysis investigated the impact of achieving early landmark responses with ponatinib on long-term outcomes in PACE pts.

**Methods:** Ponatinib treated CP-CML pts in PACE with valid cytogenetic and molecular assessments was included. Pts who met response criteria at entry, were missing assessments or not evaluable (<20 [13] metaphases examined for CCyR [MCyR]) at time of response assessment, who dropped out or who progressed (for PFS analysis) prior to response assessment were excluded. Pts were classified by molecular status (BCR-ABL<sup>IS</sup>: ≤0.1% [MMR], ≤1%, ≤10% and >10%) and cytogenetic status (MCyR, <35% Ph+ metaphases; CCyR, 0% Ph+ metaphases) at 3 and 6 months. These landmark responses were correlated with long-term outcomes; namely, PFS, OS, and molecular response over time (MMR, MR4 [BCR-ABL<sup>IS</sup> ≤0.01%], MR4.5 [BCR-ABL<sup>IS</sup> ≤0.0032%]). The log-rank test was performed to compare pts who met the criteria for response versus pts who did not meet the criteria for response at the landmark with regard to PFS and OS. Data are as of Jan 6 2014. The median follow-up was 27.9 (0.1-39.5) months.

**Results:** 267 CP-CML pts were included in the analysis: 54% male; median age, 60 (18-94) years; median time from diagnosis, 7 (0.5-27) years. Pts were heavily pre-treated: 60% received ≥3 TKIs. At 3 months, 51%, 36%, and 15% pts had reached ≤10% BCR-ABL<sup>IS</sup>, ≤1% BCR-ABL<sup>IS</sup>, and MMR, respectively. Pts who achieved each of these responses at 3 months were significantly more likely to have improved PFS after 2 years compared with those who did not (**Table**). The trend was similar for OS: specifically, pts who reached ≤1% BCR-ABL<sup>IS</sup> or MMR at 3 months had a significantly increased likelihood of OS after 2 years. Moreover, 3-month BCR-ABL<sup>IS</sup> levels correlated with achievement of deeper molecular response (MR4 and MR4.5) over time: pts with low BCR-ABL<sup>IS</sup> levels (≤1%) were more likely to achieve MR4 or MR4.5 compared with those having higher BCR-ABL<sup>IS</sup> levels (>10%). Furthermore, pts who achieved MCyR or CCyR at 3 months were significantly more likely to have improved PFS after 2 years compared with those who did not. This trend was similar for OS: achievement of MCyR or CCyR at 3 months was associated with an increased likelihood of OS after 2 years (**Table**). Similar trends were observed for 6-month molecular and cytogenetic landmark analyses (**Table**).

**Conclusions:** In these refractory CML pts, the rapid and deep reduction in BCR-ABL levels achieved with ponatinib translated into improved long-term outcomes. These data validate the usefulness of assessing BCR-ABL levels at early time points as a goal of therapy with ponatinib since achieving early landmark response appears to be a strong predictor of better long-term outcomes.

Impact on 2-Year Outcomes*				
3-month Molecular				
n	≤10% BCR-ABL <sup>IS</sup>	n	>10% BCR-ABL <sup>IS</sup>	P-value <sup>a</sup>
82	PFS = 76%	79	PFS = 57%	0.0366
86	OS = 85%	89	OS = 86%	0.6182
3-month Molecular				
n	≤1% BCR-ABL <sup>IS</sup>	n	>1% BCR-ABL <sup>IS</sup>	P-value <sup>a</sup>
75	PFS = 84%	133	PFS = 59%	0.0001
77	OS = 90%	148	OS = 84%	0.0507
3-month Molecular				
n	MMR	n	No MMR	P-value <sup>a</sup>
32	PFS = 96%	180	PFS = 64%	0.0035
33	OS = 96%	197	OS = 84%	0.0402
3-month Cytogenetic				
n	MCyR	n	No MCyR	P-value <sup>a</sup>

97	PFS = 85%	97	PFS = 52%	<0.0001
100	OS = 91%	109	OS = 81%	0.0120
<b>CCyR</b>		<b>No CCyR</b>		
78	PFS = 85%	110	PFS = 59%	0.0002
80	OS = 90%	123	OS = 83%	0.0536
<b>6-month Molecular</b>				
n	<b>≤10% BCR-ABL<sup>IS</sup></b>	n	<b>&gt;10% BCR-ABL<sup>IS</sup></b>	P-value <sup>a</sup>
84	PFS = 83%	55	PFS = 51%	0.0012
88	OS = 94%	73	OS = 84%	0.0274
<b>≤1% BCR-ABL<sup>IS</sup></b>		<b>&gt;1% BCR-ABL<sup>IS</sup></b>		
95	PFS = 84%	86	PFS = 55%	0.0001
101	OS = 93%	106	OS = 87%	0.1415
<b>MMR</b>		<b>No MMR</b>		
57	PFS = n/a	128	PFS = 60%	0.0002
61	OS = 95%	150	OS = 87%	0.0580
<b>6-month Cytogenetic</b>				
n	<b>MCyR</b>	n	<b>No MCyR</b>	P-value <sup>a</sup>
116	PFS = 72%	57	PFS = 51%	<0.0001
118	OS = 91%	73	OS = 86%	0.1051
<b>CCyR</b>		<b>No CCyR</b>		
95	PFS = 84%	75	PFS = 51%	<0.0001
97	OS = 92%	91	OS = 86%	0.1676

\*PFS and OS based on Kaplan-Meier estimates, and calculated from landmark time point. Progression defined as death, development of AP or BP, loss of CHR (in absence of cytogenetic response), loss of MCyR or increasing WBC without CHR

<sup>a</sup>Log-rank test

## 155 Defining Therapy Goals for Major Molecular Remission in Chronic Myeloid Leukaemia: Results of the Randomized CML-Study IV

<https://ash.confex.com/ash/2014/webprogram/Paper69967.html>

**Background:** In the current ELN recommendations (Baccarani et al., Blood 2013) the optimal time point to achieve major molecular remission (MMR) is defined at 12 months after diagnosis of CML. MMR is not a failure criterion at any time point leading to uncertainties when to change therapy in CML patients not reaching MMR after 12 months.

**Aims:** We sought to evaluate a failure time point for MMR using data of the CML-Study IV, a randomized five-arm trial designed to optimize imatinib therapy alone or in combination. In addition the optimal time-point to achieve a MMR should be evaluated.

**Methods:** Patients with valid molecular analysis on MR<sup>4</sup> level were divided randomly into a learning (LS) and a validation sample (VS). For the LS, MR<sup>2</sup> (defined as BCR-ABL<1% which corresponds to complete cytogenetic remission (Lauseker et al. 2014)), MMR and deep molecular remission levels (MR<sup>4</sup> or deeper) monthly landmarks were defined between one and five years after diagnosis. A patient was considered to be in MR<sup>2</sup>, MMR or MR<sup>4</sup> from the first diagnosis of the corresponding remission level and could only change to a higher level of response. Patients were censored after SCT. The best prediction time was found via “dynamic prediction by landmarking” (van Houwelingen, Scand J Stat 2007). For the failure time point analysis, for each of the resulting 48 landmarks, a Cox model was used to define the time to



progression with age and EUTOS score as additional prognostic factors. Additionally, the regression coefficients of the model of one landmark were converted to hazard ratios (HR) and treated as dependent on the HRs of the other landmarks, using a cubic smoothing function (see Fig 1). The minimum of this function was considered to be the optimal landmark point for the prediction of progression-free survival (PFS). For the calculated time point, landmark analysis for probability of PFS (defined as appearance of accelerated phase, blast crisis or death) was performed in the VS. For the evaluation of the optimal time point of achieving a MMR the same analysis was done from 0.25 to 5 years to define the time to MR4 or deeper.

*Results:* 1551 patients were randomized from 2002 to 2012, 1358 had a valid molecular analysis on the MR<sup>4</sup> level. 114 patients in the imatinib after IFN arm and 16 patients with missing EUTOS score were excluded. Of the 1228 evaluable patients two thirds were randomly allocated to the LS (n=818) and one third to the VS (n=410). Percentage of patients of the LS in MR<sup>2</sup>, MMR and MR<sup>4</sup> or deeper at one year was 28%, 29% and 14%, and at 5 years 5%, 21% and 71%, respectively. Monthly time points in between were also calculated. 44 patients of the LS reached MMR on second generation tyrosine kinase inhibitors.. The minimum of the cubic function of the HRs was found for MMR at 2.34 years with a HR of 0.25 (compared to patients without any remission) and 0.75 compared to those in MR<sup>2</sup>. For MR<sup>4</sup> or deeper no exact time point could be calculated (see Fig. 1), although it was shown that the risk of progression was slightly lower for MR<sup>4</sup> than for MMR. Since the time interval for molecular evaluation in the study is 3 months, the validation was done with 2.25 instead of 2.34 years. 364 of the 410 of the VS were still at risk at this time point and evaluable. A significant PFS advantage for patients in MMR could be demonstrated (p=0.018). At 8 years, the probability of PFS for patients in MMR was 90.8% (confidence interval 87.0-93.7%) vs. 80.5% (confidence interval 70.2-88.6%) for patients not in MMR (see Fig 2).

For the optimal MMR analysis no singular time point could be calculated as the earlier a MMR was reached the higher was the chance to achieve a MR4.

*Conclusions:* In this model, an optimal time point to predict PFS in patients with MMR was defined at 2.25 years after diagnosis and could be validated as significant. Nevertheless, patients being in MMR had a lower risk of progression than patients not being in MMR on any other time point as well. With this model we can give hints when to define MMR as failure and a change in therapy should be considered. Despite this we should keep in mind that the earlier MMR was achieved the higher was the chance to achieve deep molecular response later during therapy.

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## 520 Seven-Year (yr) Follow-up of Patients (pts) with Imatinib-Resistant or -Intolerant Chronic-Phase Chronic Myeloid Leukaemia (CML-CP) Receiving Dasatinib in Study CA180-034, Final Study Results

<https://ash.confex.com/ash/2014/webprogram/Paper67340.html>

**Background:** Dasatinib is a potent BCR-ABL tyrosine kinase inhibitor (TKI) currently approved at 100 mg once daily (QD) as a first-line therapy in CML-CP pts and a second-line therapy in pts with CML resistant/intolerant to prior therapy. CA180-034 (NCT00123474), a prospective, randomized phase 3 study, was designed to compare the dose and schedule of dasatinib therapy for the optimal benefit/risk ratio among pts with imatinib-resistant or -intolerant CML-

CP. Results from this study have previously demonstrated significant efficacy of dasatinib in this pt population. Here, we report the final 7-yr analysis of efficacy and safety outcomes of CA180-034, which represents the longest follow-up of any second-generation BCR-ABL TKI to date.

**Methods:** The CA180-034 2 X 2 factorial study design has previously been described (Shah 2010, *J Clin Oncol*). Pts (n=670) were randomized to dasatinib: 100 mg QD (n=167), 50 mg twice daily (BID; n=168), 140 mg QD (n=167), or 70 mg BID (n=168). To manage inadequate response or adverse events (AEs), dose escalation (up to a total daily dose [TDD] of 180 mg) and dose interruption or reduction (down to a TDD of 20 mg) were allowed. After 2 yrs, the protocol was amended to allow switching to a QD regimen with the same TDD after at least one dose reduction for recurrent anaemia, thrombocytopenia, neutropenia, pleural effusion, or any other fluid retention during study progress or at the investigator's discretion (Shah 2014, *Blood*).

**Results:** Approximately 55% (50 mg BID) and 51% (70 mg BID) of pts treated after the protocol amendment switched to QD dosing by the last recorded dose. The overall median duration of therapy was longer for the 100 mg QD group (37.4 months [mos]) compared with the 50 mg BID, 140 mg QD, and 70 mg BID groups (28.1 mos, 26.6 mos, and 28.9 mos, respectively). At 7 yrs of follow-up, progression-free survival (PFS) and overall survival (OS) rates were similar for all doses, as were the proportions of pts with a best on-study molecular response of MMR (Table 1). In an exploratory landmark analysis, pts in the 100 mg QD arm with BCR-ABL  $\leq 10\%$  (on the International Scale) at 3 mos had improved PFS and OS rates at 7 yrs relative to pts with BCR-ABL  $> 10\%$  (Table 2). BCR-ABL mutations were assessed in pts prior to the start of dasatinib (baseline), at the time of disease progression, or at end of treatment. Three mutations persisted or developed in pts who discontinued dasatinib due to loss of response on 100 mg QD: V299L (n=3), T315I (n=6), and F317L (n=7). For 100 mg QD, most nonhematologic and hematologic AEs (all grades) typically first occurred within the first 24 mos of treatment. Rates of nonhematologic AEs (all grades) over 7 yrs for 100 mg QD compared with other treatment arms included fluid retention (51% vs 54%), diarrhoea (42% vs 47%), nausea/vomiting (27% vs 43%), myalgias/arthralgias (38% vs 33%), fatigue (37% vs 34%), and rash (33% vs 36%). Within yr 7 of the study, new cases of pleural effusion occurred in 5% (2/42) of pts at risk treated with dasatinib 100 mg QD compared with 8% (7/88) in other treatment arms. Severe (grade 3–4) AEs (any relationship) occurred less frequently in the 100 mg QD group (98/165, 59%) relative to other treatment arms (341/497, 69%). Three pts died due to study drug toxicity (1 due to sepsis; 1 due to pulmonary oedema, congestive heart failure, neck pain, and pleural effusion; 1 due to necrosis of the colon).

**Conclusions:** Long-term follow-up of dasatinib continues to demonstrate durable efficacy and benefit for pts with CML-CP following imatinib therapy, particularly if achieving BCR-ABL  $\leq 10\%$  at 3 mos. Dasatinib is well-tolerated amongst pts, with most AEs occurring early on during the course of treatment; however, pleural effusion did occur through 7 yrs of treatment. No new safety signals were detected.

Table 1. Efficacy results

	Patients			
	100 mg QD (n=167)	50 mg BID (n=168)	140 mg QD (n=167)	70 mg BID (n=168)
MMR in assessed treated patients, n (%)	73 (46)	70 (44)	68 (44)	69 (46)
PFS at 7 yrs, % (95% CI)	42 (33–51)	44 (35–53)	38 (30–47)	44 (35–52)
OS at 7 yrs, % (95% CI)	65 (56–72)	70 (62–77)	73 (65–80)	68 (60–75)

Table 2. Landmark analysis: Efficacy rates at 7 yrs

	Patients 100 mg QD (n=165)	
	BCR-ABL $\leq$ 10% at 3 mos	BCR-ABL > 10% at 3 mos
PFS, % (95% CI)	56 (43–67)	21 (10–34)
OS, % (95% CI)	72 (60–81)	56 (42–68)
Transformation-free, <sup>a</sup> % (95% CI)	93 (84–97)	88 (67–96)

<sup>a</sup> Limited to events during the study therapy as reason for progression not collected in follow-up.

## 1796 Deep Molecular Response in Patients with Newly Diagnosed Chronic Myeloid Leukaemia in Chronic Phase (CML-CP) Treated With Nilotinib: ENESTnext Update

<https://ash.confex.com/ash/2014/webprogram/Paper70747.html>

**Background:** The BCR-ABL tyrosine kinase inhibitor nilotinib elicits faster and deeper molecular responses (MRs) vs imatinib in patients with CML-CP. Achievement of sustained deep MR is associated with improved long-term outcomes and is a key criterion for entry into treatment-free remission (TFR) studies. Given the importance of accurately measuring deep MR in patients with CML, increasingly sensitive techniques are needed for monitoring minimal residual disease. In ENESTnext, MR to nilotinib was assessed using conventional methodology (real-time quantitative reverse transcriptase polymerase chain reaction [RQ-PCR]) and a novel microfluidic digital PCR assay that is > 1 log more sensitive than standard RQ-PCR.

**Methods:** In this single-arm, open-label, multicentre study (NCT01227577), adults with CML-CP diagnosed within 6 months of enrolment were treated with nilotinib 300 mg twice daily (BID) for up to 2 years. Dose escalation to nilotinib 400 mg BID for patients with suboptimal response or treatment failure (per modified European LeukemiaNet 2009 recommendations) was permitted per physician discretion. RQ-PCR evaluation of peripheral blood samples was performed by a central laboratory (monthly for the first 3 months and every 3 months thereafter) according to the International Scale (IS). The primary endpoint is the rate of confirmed ( $\geq 2$  samples taken 3 months apart) MR<sup>4.5</sup> ( $\geq 4.5$ -log reduction of BCR-ABL transcript levels; BCR-ABL<sup>IS</sup>  $\leq 0.0032\%$ ) with 2 years of nilotinib therapy; complete cytogenetic response (CCyR) and major MR (MMR; 3-log reduction of BCR-ABL transcript levels; BCR-ABL<sup>IS</sup>  $\leq 0.1\%$ ) were evaluated as secondary endpoints. Per protocol, assessment of cytogenetic response was not required at specified time points for all patients on study. In an exploratory analysis, samples from patients with confirmed MR<sup>4.5</sup> by conventional RQ-PCR were also evaluated using the more sensitive Fluidigm digital PCR platform. The data cut-off date for this analysis was April 30, 2014.

**Results:** A total of 128 patients were enrolled (median age, 56.5 years [range, 21.0–89.0 years]); 64 patients (50.0%) were male and 103 (80.5%) were Caucasian. As of the data cut-off, 45 patients (35.2%) had completed the study, 49 (38.3%) remained on treatment, and 34 (26.6%) had discontinued early. With a median treatment duration of 12.7 months, 88 (68.8%), 94 (73.4%), and 32 (25.0%) patients achieved CCyR, MMR, and MR<sup>4.5</sup>, respectively, at any time (Table). Of 32 patients who achieved MR<sup>4.5</sup>, 14 achieved MR<sup>4.5</sup> by 6 months. A total of 169 samples from 32 patients with confirmed MR<sup>4.5</sup> by conventional RQ-PCR were analysed by digital

PCR. Using the digital PCR platform, 6 of these patients initially had detectable BCR-ABL transcripts that subsequently became undetectable with continued nilotinib therapy. Of the remaining 26 patients, 12 had BCR-ABL transcripts that were initially undetectable and remained undetectable by digital PCR, 12 had detectable BCR-ABL transcripts that remained detectable, and 2 had undetectable BCR-ABL transcripts that became detectable. The most common ( $\geq 4$  patients) grade 3/4 adverse events (AEs) regardless of relationship to study drug were increased lipase ( $n = 14$ ), thrombocytopenia ( $n = 11$ ), neutropenia ( $n = 8$ ), hypophosphatemia ( $n = 5$ ), anaemia ( $n = 4$ ), and nausea ( $n = 4$ ). Reasons for study discontinuation were AEs ( $n = 15$ ), unsatisfactory therapeutic effect ( $n = 5$ ), withdrawn consent ( $n = 4$ ), death ( $n = 3$ ; causes of death were other malignancy, pneumonia, and not specified/no AE [ $n = 1$  each]), protocol deviation ( $n = 3$ ), abnormal laboratory values ( $n = 2$ ), loss to follow-up ( $n = 1$ ), and administrative problems ( $n = 1$ ).

**Conclusions:** Frontline treatment with nilotinib 300 mg BID in patients with newly diagnosed CML-CP led to rapid achievement of MR<sup>4.5</sup> as assessed with conventional RQ-PCR. As  $> 40\%$  of samples with at least MR<sup>4.5</sup> according to standard RQ-PCR were positive using the digital PCR assay, this tool may have potential in evaluating MR to determine eligibility for TFR studies.

**Table**

	<b>Response</b>		
	<b>CCyR</b>	<b>MMR</b>	<b>MR<sup>4.5</sup></b>
Patients with response, n (%)	88 (68.8)	94 (73.4)	32 (25.0)
Time to response, n (%)			
< 3 mo	26 (20.3)	21 (16.4)	2 (1.6)
3 to < 6 mo	42 (32.8)	41 (32.0)	12 (9.4)
6 to < 12 mo	16 (12.5)	22 (17.2)	11 (8.6)
12 to < 18 mo	4 (3.1)	9 (7.0)	7 (5.5)
$\geq 18$ mo	0	1 (0.8)	0

<sup>a</sup> Cytogenetic response was not assessed in all patients at all time points.

## 1802 Propensity Score Matched Comparison of Dasatinib and Nilotinib as a Frontline Therapy in Newly Diagnosed CML with Chronic Phase

<https://ash.confex.com/ash/2014/webprogram/Paper75039.html>

**Background:** Dasatinib (DAS) and nilotinib (NIL) are standard frontline therapy for chronic myeloid leukaemia, chronic phase (CML-CP) based on randomized trials compared to imatinib. However, DAS and NIL have not been compared directly. The purpose of this study is to analyze efficacy, long-term outcome and toxicity of DAS and NIL as a front line therapy in newly diagnosed CML-CP.

**Method:** Newly diagnosed patients (pts) with CML-CP, who received front-line therapy by either one of the phase II trials conducted almost in parallel (DAS: NCT00254423, N = 102 and NIL: NCT00129740, N = 104) are matched with caliper matching by the propensity score (PS) to

adjust pre-treatment confounding factors. DAS was given orally by either 50mg twice daily (N = 30) or 100mg daily (N = 77). NIL was given 400mg orally twice daily. Toxicity was recorded according to the CTCAE ver. 4.0.

**Result:** PS matching resulted in 87 pts from each trial to be matched for pre-treatment characteristics including age, Sokal score, lab, and organ function (Table). The median observation duration was 50.9 months (95% CI: 40.1-61.7) vs. 43.0 months (95% CI: 35.3-50.7) (DAS vs. NIL, P = 0.56). Response rate at 3, 6, and 12 months as well as cumulative (best) response are shown in Table. There were no significant differences in measures of response throughout the study period except for a higher rate of complete molecular response at 6 months with NIL (NIL vs. DAS, 11% vs. 3%, P = 0.04). However, at 12 months, this difference was not retained; there was also no difference in the rate of optimal response at 3 months. There was no statistical difference in cumulative response between 2 groups. No statistical difference was observed between 2 groups in any of the survival endpoints at 3 years (overall, event-free, failure-free, and transformation-free survival). Treatment discontinuation was observed in 16 (18%) vs. 17 (19%) pts with (DAS vs. NIL, P = 0.82). Reason for the discontinuation was; 1) toxicity (8 vs. 8, P = 1.00), 2) resistance (5 vs. 8, P = 0.39), and 3) financial (4 vs. 1, P = 0.37) (all presented as DAS vs. NIL, respectively). Adverse event (AE) was observed in 40 (46%) vs. 42 (48%) pts (DAS vs. NIL, P = 0.76), whereas grade 3 or more AE was observed in 19 (22%) vs. 15 (17%) pts (DAS vs. NIL, P = 0.44).

**Conclusion:** In PS matched cohort of newly diagnosed CML-CP pts, the outcome observed with both treatment options (DAS and NIL) is excellent with no clear difference in response or long-term survival endpoints. Incidence of clinically significant AEs was similar between DAS and NIL.

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## 398 ABL001, a Potent Allosteric Inhibitor of BCR-ABL, Prevents Emergence of Resistant Disease When Administered in Combination with Nilotinib in an *in Vivo* Murine Model of Chronic Myeloid Leukaemia

**Background:** Chronic myelogenous leukaemia (CML) and a subset of acute lymphoblastic leukaemia (ALL) are caused by the t(9;22)(q34;q11.2) chromosome translocation, resulting in fusion of the BCR and ABL1 genes on the Philadelphia chromosome to encode constitutively active ABL1 kinase. Despite the dramatic progress made over the past decade with tyrosine kinase inhibitors (TKIs) in the treatment of CML, allogeneic stem cell transplant is considered the only proven curative therapy. To achieve cure or benefit from treatment-free remissions with pharmacologically-based therapies, it is estimated that patients will likely need to achieve a sustained reduction in tumour burden of 4 logs (MR<sup>4</sup>) or deeper (MR<sup>4.5</sup>). Currently, only 39% and 18% of patients achieve MR<sup>4</sup> by 24 months of treatment with single agent nilotinib or imatinib, respectively. Furthermore, for a subset of CML patients and the majority of Ph+ ALL patients, resistance develops to current TKI's as a result of emergence of point mutations in the ATP site of the kinase domain. ABL001 is a potent, selective BCR-ABL inhibitor that maintains activity across most mutations, including T315I, with a distinct, allosteric mechanism of action which recently entered Phase I development for the treatment of patients with CML and Ph+



ALL. ABL001 was developed to be dosed in combination with nilotinib to provide greater pharmacological coverage of BCR-ABL disease and prevent the emergence of resistance.

*Methods:* Based on X-ray crystallography, NMR and molecular modelling, ABL001 is the result of a structure-guided medicinal chemistry program targeting the myristoyl pocket of the ABL1 kinase. *In vitro* cell based assays were performed using the Ba/F3 isogenic cell system and a panel of over 300 cell lines. KCL-22 cells were used to develop an *in vivo* xenograft model to assess the efficacy of ABL001 and the PD marker, pSTAT5, was used to monitor the inhibition of BCR-ABL signalling.

*Results:* In contrast to TKIs that bind to the ATP-site of the ABL1 kinase domain, NMR and X-Ray crystallography studies confirmed that ABL001 binds to a pocket on the BCR-ABL kinase domain that is normally occupied by the myristoylated N-terminus of ABL1. Upon fusion with BCR, this myristoylated N-terminus that serves to autoregulate ABL1 activity is lost. ABL001 functionally mimics the role of the myristoylated N-terminus by occupying its vacant binding site and restores the negative regulation of the kinase activity. Cell proliferation studies demonstrate that ABL001 selectively inhibited the growth of CML and Ph+ ALL cells with potencies ranging from 1-10nM range. In contrast, BCR-ABL-negative cell lines remained unaffected at concentrations 1000-fold higher. With resistance emerging in the clinic to current TKI's as a result of point mutations in the ATP-site, ABL001 was tested for activity against clinically observed mutations and found to be active in the low nM range. In the KCL-22 mouse xenograft model, ABL001 displayed potent anti-tumour activity with complete tumour regression observed and a clear dose-dependent correlation with pSTAT5 inhibition. The KCL-22 xenograft model was also used to compare the dosing of ABL001 and nilotinib as single agents to dosing a combination of ABL001 and nilotinib. Single agent dosing regimens led to tumour regressions; however, despite continuous dosing, all tumours relapsed within 30-60 days with evidence of point mutations in the resistant tumours. In contrast, animals treated with the combination of ABL001 and nilotinib achieved sustained tumour regression with no evidence of disease relapse either during the 70 days of treatment or for > 150 days after treatment stopped.

*Conclusion:* ABL001 selectively inhibited the proliferation of cells expressing the BCR-ABL fusion gene and was active against clinically important mutations that arise with current TKI therapy in CML. In an *in vivo* model of CML, the combination of ABL001 and nilotinib resulted in complete and sustained tumour regression with no evidence of disease relapse. These results provide proof-of-principle that simultaneous targeting of the myristoyl pocket and ATP-pocket by ABL001 and nilotinib, respectively, promotes a more sustained overall efficacy and prevents the emergence of resistance via acquisition of point mutations in the respective binding sites. ABL001 is currently being evaluated in a Phase 1 study in patients with CML and Ph+ ALL.

**\*\*See below for information about related Phase 1 trial currently open and recruiting in US, France, Germany, Italy, Spain, Netherlands, Japan, Korea, Singapore and Australia.**

A Phase I, Multicentre, Open-label Study of Oral ABL001 in Patients with Chronic Myelogenous Leukaemia (CML) or Philadelphia Chromosome-positive Acute Lymphoblastic Leukaemia (Ph+ ALL)

<http://clinicaltrials.gov/ct2/show/NCT02081378?term=ABL001&rank=1>

#### Detailed Description:

This first-in-human trial with ABL001 is a dose escalation study whose primary purpose is to estimate the maximum tolerated dose (MTD) and/or recommended dose for expansion (RDE) of ABL001 administered orally as a single agent to adult patients with CML or Ph+ ALL. The safety, tolerability and pharmacokinetic (PK) profile of ABL001 will be assessed together with an evaluation of pharmacodynamics (PD) changes in peripheral blood mononuclear cells (PBMC) and bone marrow aspirates and all data may contribute to the assessment of the RDE.

An understanding of the MTD/RDE, safety profile, PK/PD relationship, and preliminary evidence of anti-CML and ALL activity will be used to inform future development in adults with CML and Ph+ ALL. By virtue of its distinct pharmacological profile and by preclinical pharmacological studies demonstrating an additive effect, a combination of ABL001 and a tyrosine-kinase inhibitor (TKI) has the potential to achieve a deeper molecular response in a higher proportion of CML patients as compared to single agent TKI therapy. Such a combination has the added advantage of targeting the ABL kinase domain at two distinct locations, theoretically preventing single point mutation-associated treatment resistance. The prediction is that a nilotinib/ABL001 combination will increase the percentage of patients who achieve a complete molecular response (CMR) and decrease the time to CMR, thereby increasing the possibility of achieving sustained treatment-free remissions in these patients. In addition, some patients may be intolerant of therapy with TKIs or may develop mutations that promote resistance to TKI therapy. In these patients, ABL001 may provide a novel therapeutic option.

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## 399 Detection of *BCR-ABL1* Compound and Polyclonal Mutants in Chronic Myeloid Leukaemia Patients Using a Novel Next Generation Sequencing Approach That Minimises PCR and Sequencing Errors

<https://ash.confex.com/ash/2014/webprogram/Paper74252.html>

### **Background**

*BCR-ABL1* kinase domain (KD) mutations are the most common known cause of resistance to tyrosine kinase inhibitors (TKIs) in CML. Mutation analysis is critical for selection of subsequent TKI therapy after treatment failure. Low level and compound mutants (>1 KD mutation in the same molecule) may also lead to therapy failure. However, compound and multiple polyclonal mutants cannot be distinguished by conventional methods as they determine the average genotype of all molecules. Next generation sequencing (NGS) has the potential to sensitively detect these mutants, however sequencing and PCR errors confound the detection of true, low level mutants using current approaches. Indeed, we demonstrated that the reported frequency of *BCR-ABL1* compound mutants may be overestimated due to PCR recombination artefacts that

mimic compound mutations (Parker Blood 2014). More reliable methods are needed to appropriately assess the impact of various mutations on patient (pt) outcome.

### **Aim**

To develop a clinically applicable NGS assay that can robustly distinguish *BCR-ABL1* compound and polyclonal mutants.

### **Method**

We have developed a novel NGS assay termed Single Molecule Consensus Sequencing (SMCS) that involves tagging individual *BCR-ABL1* cDNA molecules before library amplification, enabling identification and elimination of most PCR and sequencing errors. NGS was performed on the Illumina MiSeq, 2 x 300 bp; aa 244 - 407 of the KD was examined. Reads derived from an initial *BCR-ABL1* molecule are identified bioinformatically by virtue of sharing the same tag sequence. The consensus sequence of reads with the same tag is determined using automated variant calling and filtering algorithms. The consensus sequence represents the sequence of the initial *BCR-ABL1* cDNA molecule (Fig A).

### **Results**

To test the validity of SMCS, we examined 10 samples lacking KD mutations and 5 mock samples created by mixing compound mutant plasmids or pt samples. Examination of raw sequencing reads revealed a complex spectrum of mutants, similar to previous clinical reports. SMCS enabled bioinformatics filtering of these artefacts, largely eliminating PCR and sequencing error, and exclusively reported the compound and polyclonal mutants known to be present in the mock samples. We estimated the background error rate to be  $\sim 2 \times 10^{-5}$  per base. The error spectrum was consistent with DNA damage causing first round PCR errors.

SMCS was used to retrospectively examine samples of 46 pts (36 CP, 2 AP, 8 BP) who were resistant to  $\leq 4$  TKIs (1<sup>st</sup> and 2<sup>nd</sup> generation). 71 mutations were previously detected by Sanger sequencing in these samples, collected before starting next line TKI. Within the region examined using SMCS, there was 100% detection concordance with Sanger sequencing.

We compared the results of SMCS with an amplicon NGS method performed at another centre for 24/46 pts (Ion Torrent, depth  $\sim 10000$ ). Ion Torrent detected 34 compound mutants in 24 pts. Of the 30/34 that were within the region examined by SMCS we only detected 8. Based on observations in Parker Blood 2014, 14 of the 22 compound mutants not detected by SMCS were likely to be PCR recombination artefacts. The other 8/22 were low level (1 - 4%) and most (6/8) involved mutations rarely/never reported in TKI resistant pts so may also be artefacts (Fig B). We detected 3 additional compound mutants in these 24 pts, plus 5 in the remaining 22/46 pts. The compound mutants detected by SMCS were consistent with the pts' TKI treatment history.

### **Conclusion**

We demonstrated detection of *BCR-ABL1* compound and polyclonal mutants in pt samples using a novel NGS assay that has the potential to overcome technical artefacts generated with other published methods. Whilst there is no gold standard method that can accurately detect low level compound mutations, SMCS has correctly identified sequencing and PCR recombination artefacts using mock samples. The accuracy and clinical utility of SMCS for sensitive compound and polyclonal mutant detection is currently being validated in another group of 200 imatinib resistant pts. The frequency of compound mutants detected in pts with  $>1$  mutation by SMCS in the current analysis (35%) is approximately half of that reported previously, which suggests the published frequency may have been overestimated. Our novel assay takes an important step towards enabling a more concrete understanding of the mutation spectra in pts and their association with resistance.

