Abstracts and notes on CML presentations[1]
ASH 2011 San Diego

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1 Highlights

If you're pushed for time read the first two sides and you'll get the key points. I have not aimed to review all the abstracts, rather pick up and expand on some key (highly selected) themes. I have focused mainly on the oral presentations (36 of them) and clinically relevant studies. Complete abstracts are included for all oral presentations and just listings for posters. I've taken care to ensure the accuracy of the data but when furiously typing during sessions I can't always guarantee complete precision!

Abstracts are available on line at: http://ash.confex.com/ash/2011/webprogram/meeting.html

If I had to highlight three things of most relevance to practising clinicians they would be:

1. ***The '10% thing'…***
2. ***Stopping TKIs now including second generation***
3. ***Ponatinib and other drugs***

1. The '10% thing'…

An increasing body of data supports the idea that the level of BCR-ABL PCR at 3 months can predict for eventual outcome in patients treated with imatinib. Data from the German group [783] indicate that in the 28% of patients on imatinib who fail to achieve a PCR level of <10% the 5 year overall survival is 87% - still pretty good. However in the majority who do fall to levels of <10% at 3 months OS at 5 years was 7% better at 94% - a small but statistically significant difference. It could be that strategies to identify patients with a poorer response and switch them early to another treatment could become more common in the management of CML. Maybe imatinib is good enough for most patients (especially as it comes off patent in 2015 and the price will fall) and second generation drugs should be used selectively. Further trials required and time will tell.

Data from the SPIRIT 2 trial dasatinib arm were presented for the first time [785] and other 'early response' dasatinib data were also presented [2767]. 8.6% of patients on dasatinib failed to achieve the 10% threshold (c.f. 28% on imatinib from German data). Using a method to identify a more discriminatory value for segregating

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the data (ROC curve) a threshold of e.g 0.57% BCR-ABL/ABL ratio was predictive of the eventual achievement of CMR. It's too early to correlate with survival outcomes as yet. It may be that '10% at 3 months' comes into more common use as it's simple but further work might be required to define optimum thresholds to predict certain outcomes. This concept of early definition of good/less good response seems to be backed up by increasingly robust data now.

2. Stopping TKIs now including second generation

An update of the French STIM study [603] confirmed that i) about 40% of patients remain in complete molecular response (CMR) in the longer term having stopped taking imatinib; ii) most of those who relapse with increasing PCR levels do so within the first 6 months; iii) those who relapsed responded again to imatinib when reintroduced and there have been no disease progressions.

For the first time, data were presented on stopping 2nd generation drugs (dasatinib and nilotinib) [604]. Data on 33 patients were presented (18 das, 15 nil) and with 9 months of follow up only 8 have lost their CMR, all again within 6 months of stopping. So 75% of patients remain in CMR – significantly higher that the 40% comparative figure with imatinib. Caution is required: it's a small number of patients and follow up is short. However the data are very interesting and offer the possibility with the newer agents that no only could a higher level of CMR be obtained but possibly more patients could maintain that CMR after stopping drug. Further research is required to explore this and the presenters were at pains to emphasise that stopping should only be done in the context of a clinical trial with close monitoring.

As patients with excellent responses become less tolerant of low grade toxicities as the years go by the question of 'how much is enough' for all of the TKIs will become increasingly relevant.

3. Ponatinib and other drugs

There are now many more patients on **ponatinib** in clinical trials and for the first time results from a substantial number of patients on the drug were reported [109, 602]. Data on 217 patients with chronic phase CML were presented: 64 patients had the really tough T315I mutation and 94% of patients had received *two* previous TKIs so quite a tough group of patients. The complete cytogenetic response (CCR) rate overall was 39%, 33% in the resistant/intolerant group and impressively 58% in the T315I group. One of the commonest toxicities was rash/dry skin but most patients tolerated the drug fairly well. 2/3 of patients stayed on treatment at the time of analysis. So, it looks pretty good and we'll have to now figure out what it's role is in the overall management of CML. A first lines study is planned and it will still be a while before it's licensed and more broadly available.

There was a 3 year update on **nilotinib** [452]. Still continues to look good and key messages are: i) rate of MMR is significantly higher than with imatinib; ii) small difference in rates of progression: ~1% nil vs ~4% imat; iii) no difference in survival. So to my mind, further follow required to be convincing about superiority and it may well be the benefit of 2G drugs is the ability to eventually stop (see above). More time, follow up required.

Bosutinib is struggling a little [455, 110]. It's pretty good but not obviously better than imatinib and many patients get diarrhoea. Not sure where it will end up. No update on **dasatinib** phase 3 (Dasision study) – expect that at ASCO 2012. **DCC2036** [601], a switch pocket inhibitor, looks interesting. So far only 19 patients treated and there is a little concern over neuromuscular toxicity. It works in a different way to the TKIs to data so could be one to watch.

There were of course lots of other interesting abstracts but to my mind these were the key themes clinically. In the UK we are working on developing a new study, possibly 'SPIRIT 3' that would integrate a number of these concepts to see how relevant they are in ensuring excellent survival, using the right drug for the right patient and figuring out 'how much is enough' and whether we can stop treatment in a larger proportion of patients.

People who know me will tell you I take a lot of convincing but I'm starting to think now that we have the drugs and the monitoring methods to potentially allow us to cure some patients with CML and hopefully that will apply to many more in the future. Exciting times!

2 Therapy: Resistance to Tyrosine Kinase Inhibitors [109-114]

2.1. **[109] Initial Findings From the PACE Trial: A Pivotal Phase 2 Study of Ponatinib in Patients with CML and Ph+ ALL Resistant or Intolerant to Dasatinib or Nilotinib, or with the T315I Mutation.** *Cortes. Background:* Despite progress in chronic myeloid leukemia (CML) therapy with tyrosine kinase inhibitors (TKIs), patients (pts) who fail dasatinib or nilotinib or pts with T315I mutation have no treatment options. Ponatinib is a potent, oral, pan-BCR-ABL inhibitor active against the native enzyme and all tested resistant mutants, including the uniformly resistant T315I mutation. *Methods:* The PACE trial (Ponatinib Ph+ALL and CML Evaluation) was initiated in September 2010. The objective of this international, single-arm, open-label, phase 2 trial is to establish the efficacy and safety of ponatinib. Pts with refractory CML in chronic, accelerated or blast phase (CP, AP or BP), or Ph+ acute lymphoblastic leukemia (ALL), resistant or intolerant (R/I) to dasatinib or nilotinib or with the resistant T315I mutation received 45 mg ponatinib orally once daily in one of 6 cohorts: CP R/I; CP T315I; AP R/I; AP T315I; BP/ALL R/I; BP/ALL T315I. The primary endpoints are major cytogenetic response (MCyR) for CP and major hematologic response (MaHR) for AP, BP or ALL. The trial is ongoing; projected enrollment is approximately 450. Data as of 18 July 2011 are reported. *Results:* At analysis, 403 pts were enrolled; 397 were treated and eligible. The median age was 59 (range, 18-94) years, 52% were male. Diagnoses were: CP R/I, n=188; CP T315I, 48; AP R/I, 52; AP T315I, 15; BP/ALL R/I, 51; BP/ALL T315I, 43. Median time from initial diagnosis to start of ponatinib was 6.2 years. Prior TKIs included imatinib (93%), dasatinib (85%), nilotinib (66%), and bosutinib (8%); 94% failed >2 prior TKIs, and 57% failed >3 prior TKIs. Overall, 88% had a history of resistance to dasatinib or nilotinib, and 12% were purely intolerant. Mutation status was determined centrally by MolecularMD. Overall, 106 pts had the T315I mutation. Of 291 R/I pts, 110 (38%) had non-T315I BCR-ABL mutations, most frequently F317L (10%), F359V (5%), E255K (4%), and G250E (4%). To date, 343 (85%) pts remain on therapy, 60 (15%) have discontinued (42 BP/ALL): 24 (6%) progressive disease (20 BP/ALL); 11 (3%) AE (3 pain, 3 thrombocytopenia, 1 each haemorrhage, loss of consciousness, enterocolitis, cytokine release syndrome, hepatotoxicity/pleuro-pericardial effusion after overdose); 8 (2%) died (3 related; 7 BP/ALL); 17 (4%) other. The most common drug-related AEs (≥10% any grade) were thrombocytopenia (19%; 15% grade 3/4), rash (18%), dry skin (13%), myalgia (12%), abdominal pain (11%; 3% grade 3/4), headache (11%), arthralgia (11%). Overall, 67 (17%) pts experienced at least 1 related SAE. The most common related SAEs (>5 cases) were pancreatitis 15 cases (3.7%), 5 cases each (1.2%) diarrhea, anemia, febrile neutropenia, and pyrexia. At the time of reporting, 159/397 eligible pts were evaluable for the primary endpoints. Median follow-up was 57 days. Of CP pts, 83 had an assessment at 3 months (10 at 6 months) or discontinued. In CP R/I, 25/60 (42%) attained MCyR (15 CCyR). In CP T315I, 13/23 (57%) had MCyR (11 CCyR). The overall CP MCyR rate was 38/83 (46%) (26 CCyR). Of AP, BP/ALL pts, 76 had an assessment at 1 month or later or discontinued. In AP, 17/23 (74%) R/I and 1/1 T315I pts achieved MaHR. In BP/ALL, 11/30 (37%) R/I and 6/22 (27%) T315I pts had MaHR. *Conclusion:* In this first analysis of the pivotal PACE trial, ponatinib has a favorable early safety profile, similar to that observed in phase 1, but with a lower incidence of pancreatitis. Initial response data after short follow-up indicate ponatinib has substantial anti-leukemic activity in this heavily pretreated population, and in pts with refractory T315I. These early efficacy signals replicate initial response results reported in the phase 1 setting. Updated data will be presented at the annual meeting.

NOTES ON PRESENTATION

Res or intol nilot or dasat or having a T315I
CPCML 271. AP 79 BP/ALL 94
64 CP had T315I
Focus on 271 CP. 212 were resistant
94% 2 prior tki
59% 3 TKI
67% CP had no mutations
Most common mutation was F317L
Only 25% had achieved MCR on prior TKI
MCR in 41% CP patients 79/191
65% of T315I 37/57
CCR 39% overall 33% in RI cohort 58% in T315I
Common rash and dry skin
Lipase up in 16%. Pancreatitis gd3 is 6%. Not patient had to stop
1/3 of patients had to stop treatment.

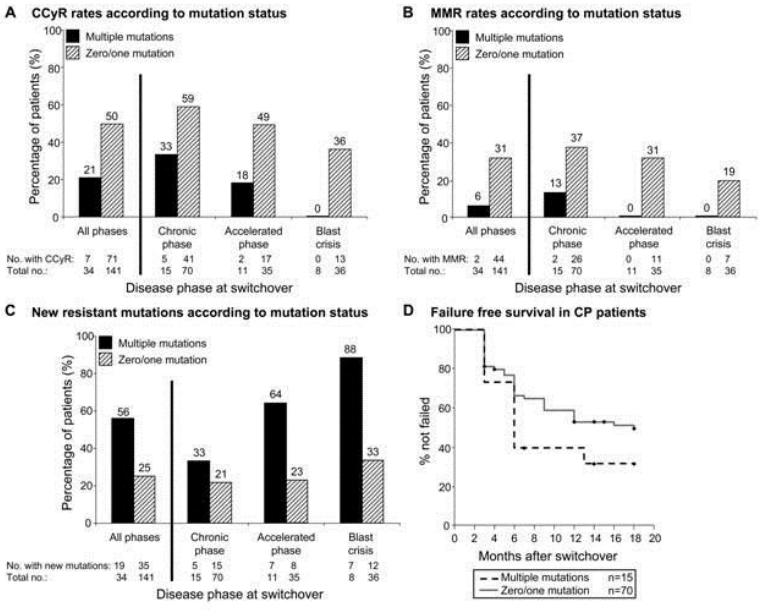
2.2. **[110] Activity of Bosutinib by Baseline and Emergent Mutation Status in Philadelphia Chromosome–Positive Leukemia Patients with Resistance or Intolerance to Other Tyrosine Kinase Inhibitors.** *Khoury* Bosutinib is an orally active, Src/Abl tyrosine kinase inhibitor (TKI) that has demonstrated clinical activity and an acceptable safety profile in an open-label, phase 1/2 study of patients (pts) with Philadelphia chromosome–positive (Ph+) leukemia following resistance or intolerance to imatinib. Abl kinase domain mutations may affect drug binding and subsequent TKI efficacy. Responses to bosutinib by Bcr-Abl baseline mutation status and the emergence of mutations during bosutinib treatment in this phase 1/2 study are reported here.A total of 570 pts in 3 cohorts received bosutinib: chronic phase chronic myeloid leukemia (CP CML) following prior imatinib only (CP2L cohort; n = 288); CP CML following prior imatinib plus dasatinib and/or nilotinib (CP3L cohort; n = 118); and advanced leukemia (ADV cohort: accelerated and blast phase CML and acute lymphoblastic leukemia; n = 164) following prior treatment with imatinib only or with other TKIs. For the CP2L, CP3L, and ADV cohorts, the respective median times since diagnosis were 3.6 y (range, 0.1-15.1 y), 6.5 y (range, 0.6-18.3 y), and 3.7 y (range, 0.1-

22.1y). Per protocol, Bcr-Abl mutational status was evaluated at baseline (pre-dose) and at the end of treatment (EOT). Mutations were assessed at baseline in 412 pts; of these, 79/212 (37%) CP2L pts, 39/83 (47%) CP3L pts, and 65/117 (56%) ADV pts had ≥1 baseline mutation, and 11 (5%), 9 (11%), and 7 (6%) pts, respectively, had ≥2 baseline mutations. The most common mutations were T315I (n = 31; prior to eligibility exclusion), F359C/I/S/V (n = 23), F317L (n = 21), G250E (n = 19), Y253F/H (n = 16), and M351T (n = 15). Rates of confirmed complete hematologic response (CHR) and major cytogenetic response (MCyR) were generally similar among chronic phase pts (CP2L, CP3L) with and without baseline mutations, although the presence of a mutation negatively impacted response rates in ADV pts (CHR, 17% vs 39%; MCyR, 24% vs 37%; see Table). CHR and/or MCyR were observed broadly across mutations, including those associated with dasatinib (F317L) or nilotinib (Y253F/H, E255K/V, and F359C/I/S/V) resistance, with the exception of the T315I mutation in all cohorts and the F317L and Y253H mutations in the ADV cohort (see Table). Of the 140 pts who were assessed for emergent mutations at treatment completion, a new mutation was identified in 39 (28%) pts. Pts with a new mutation were more likely to have discontinued treatment due to PD/unsatisfactory response (UR; n = 34/39 [87%]) than those without a new mutation (n = 47/101 [47%]). The most common emergent mutations were T315I (n = 14) and V299L (n = 12), and nearly all pts who developed these mutations (93% and 100% of pts, respectively) discontinued treatment due to PD/UR. New mutations were more common in pts with ≥1 baseline mutation (n = 26) versus those without a baseline mutation (n = 13); of those pts, 21/26 (81%) and 13/13 (100%), respectively, discontinued treatment due to PD/UR. Eight pts discontinued treatment due to PD/UR within 6 months of starting bosutinib; all but 1 patient had a T315I or V299L mutation present at baseline or identified at treatment completion. In conclusion, clinical activity to bosutinib was observed in CML pts across baseline Bcr-Abl kinase domain mutations, including those associated with resistance to other second-generation TKIs, except typically for T315I. These results support the observed benefit of bosutinib in pts with Ph+ leukemia following prior treatment with TKIs. Pts in whom new mutations were identified at treatment completion were more likely to have pre-existing mutations and to discontinue treatment due to PD/UR. The most common mutations to emerge during bosutinib therapy were T315I and V299L, some of which may have been present but not detected at baseline.

570 patients in total
V299L and T315I were most common emergent mutations on bosutinib.

2.3. **[111] Multiple Low Level Mutations Identifies Imatinib Resistant CML Patients At Risk of Poor Response to Second-Line Inhibitor Therapy, Irrespective of the Resistance Profile of the Mutations.** *Parker.* Specific imatinib resistant BCR-ABL1 mutations confer clinical resistance to nilotinib (NIL; Y253H, E255K/V, T315I, F359V/C) and/or dasatinib (DAS; V299L, T315I/A, F317L/I/V/C). Therefore, mutation analysis is recommended for CML patients (pts) after imatinib failure to facilitate selection of appropriate therapy. However, around 40% of chronic phase (CP) pts without these NIL/DAS resistant mutations also fail second line inhibitor therapy. For imatinib resistant pts without these mutations at the time of commencing NIL/DAS therapy (switchover) we investigated whether sensitive mutation analysis could identity pts at risk of poor response to subsequent therapy. Switchover samples of 220 imatinib resistant pts (DAS n=131, NIL n=89) were analysed by direct sequencing (detection limit 10-20%) and sensitive, high throughput mass spectrometry (mass spec; Sequenom MassARRAY, detection limit 0.05-0.5%), which detects 31 common BCR-ABL1 mutations (approximately 89% of mutations detected in pts receiving imatinib). We previously demonstrated that mass spec could detect NIL/DAS resistant mutations at switchover in an extra 9% of pts compared to sequencing and that these low level resistant mutations were associated with subsequent failure of these inhibitors (Parker et al, JCO. 2011 In Press). Therefore, for the current analysis, pts with NIL/DAS resistant mutations detected by either method (n=45) were excluded since response is already known to be poor in these cases. In the switchover samples of the remaining 175 pts, 159 mutations were detected in 86 pts by mass spec, but just 108 mutations were detected in 89 pts by sequencing. Thirteen rare mutations detected by sequencing were not included in the mass spec assay design. Mass spec detected all other mutations detected by sequencing, plus an additional 64 low level mutations. Multiple NIL/DAS sensitive mutations (≥2 mutations) were detected at switchover in more of the 175 pts by mass spec (34/175, 19%; 2-9 mutations per pt) than sequencing (16/175, 9%; 2-3 mutations per pt), *P*=.009. We divided pts into 2 groups; those with multiple mutations detected by mass spec at switchover (n=34) and those with 0/1 mutation (n=141), and investigated the impact of multiple mutations on response to subsequent NIL/DAS therapy. Pts with 0 or 1 mutation, and similarly pts with 2 or >2 mutations, were grouped together, as no difference in response was observed. The median follow up for CP, accelerated phase and blast crisis pts was 17 (2-33), 18 (1-33) and 3 (1-27) mo, and the frequency of multiple mutations was 18%, 24% and 18%, respectively. During follow up, multiple mutations at switchover was associated with lower rates of complete cytogenetic response (CCyR; 21% vs 50%, *P*=.003, Fig 1A) and major molecular response (MMR; 6% vs 31%, *P*=.005, Fig 1B), and a higher incidence of acquiring new NIL/DAS resistant mutations detectable by sequencing (56% vs 25%, *P*=.0009, Fig 1C). At 18 mo, the failure-free survival rate (European LeukemiaNet recommendations) for CP pts with

Figure 1. The association between multiple nilotinib/dasatinib sensitive mutations detected at switchover by multiplex mass spectrometry and response to nilotinib/dasatinib therapy after imatinib resistance in CML patients.



multiple mutations at switchover was 33% compared to 51% for CP pts with 0 or 1 mutation (*P*=.26, Fig 1D). The number of mutations detected per pt by mass spec at switchover (max of 9, 8 of 86 pts with mutations had ≥4, 9%) far exceeded the number concurrently detected by sequencing (max of 3). This suggests that mass spec detected a pool of subclonal mutants, each with a small survival advantage after imatinib therapy that was insufficient for their clonal predominance. Multiple low level mutations may be a marker of an increased propensity for subsequent selection of resistant mutations, possibly driven by genetic instability, demonstrating the advantage of a sensitive multiplex mutation assay. In conclusion, sensitive mutation analysis identified a poor-risk subgroup with multiple mutations that were not identified by sequencing. This subgroup represented 15.5% of the total cohort (34/220), who would not otherwise be classified as being at risk of poor response on the basis of their mutation status. These pts did not have NIL/DAS resistant mutations at switchover; however, they had a lower incidence of CCyR and MMR, and higher incidence of acquiring new NIL/DAS resistant mutations during NIL/DAS therapy compared to pts with 0 or 1 mutation. This poor-risk subgroup may warrant closer monitoring or experimental approaches to reduce the high risk of kinase inhibitor failure after imatinib resistance.

220 patients switching to nil or das.

2.4. **[112] Validation of the New European LeukemiaNet (ELN) Recommendations for Bcr-Abl Kinase Domain Mutation Analysis In Chronic Myeloid Leukemia: An Analysis of the GIMEMA CML Working Party Studies**. *Soverini.* BCR-ABL kinase domain (KD) mutation analysis may be an useful tool for physicians and is being performed in a growing number of laboratories. Recommendations aimed to rationalize the use of mutation testing in chronic myeloid leukemia (CML) have recently (Blood 2011) been compiled by a panel of experts appointed by European LeukemiaNet (ELN) – including specific recommendations as to when mutation analysis should be performed. They came from the expert opinion of the panel members whenever published data were insufficient or contradictory. In order to provide further data to validate or refine these recommendations, we have analyzed the GIMEMA CML WP database recording the results of mutation analyses performed in CML pts (n=1301) receiving imatinib and/or 2nd generation TKIs between January 2004 and July 2011. At dagnosis, mutation analysis was recognized to be useful in the few pts who present in accelerated phase or blast crisis (BC), while it was not recommended in chronic phase (CP) pts. Interrogating our database, we could retrieve 58 mutation analyses in newly diagnosed pts in CP and 12 in newly diagnosed pts in BC. Imatinib-resistant mutations were detected in 0 and 2 pts, respectively. In pts receiving 1st-line imatinib, mutation analysis was recommended both in case of failure and in case of suboptimal response. We have analyzed 399 chronic phase (CP) CML pts receiving first-line imatinib because they were found to meet one of the criteria for failure or suboptimal response. Overall, 45/166 (27.1%) failures were found to be positive for one or more BCR-ABL KD mutations. In particular, mutations were detected in 3/16 (18.8%) pts with less than CHR at 3 months, 1/9 (11.1%) pts with no CyR at 6 months, 4/24 (16.7%) pts with less than PCyR at 12 months, 6/36 (16.7%) pts with less than CCyR at 18 months, 15/49 (30.6%) pts who lost CCyR and 16/32 (50%) pts who lost CHR. More interestingly, only 11/233 (4.7%) suboptimal responders we analyzed were positive for mutations. Among 'cytogenetic' suboptimal responders, mutations were detected in 1/15 (6.7%) pts with no CyR at 3 months, 1/20 (5.0%) pts with less than PCyR at 6 months, 5/51 (8.2%) pts with less than CCyR at 12 months. Among 'molecular' suboptimal responders, mutations were detected in 0/52 pts with less than MMR (but having achieved CCyR) at 18 months and in 4/95 (4.2%) pts who lost MMR (but not CCyR). Which rise in Bcr-Abl transcript level should trigger a mutation analysis was the most difficult issue to provide recommendations upon, given the lack of convincing and reproducible data in the literature. It was finally agreed to recommend mutation analysis only in case of MMR loss. In 159 of the CP pts we have analyzed, mutation analysis was specifically requested because of a transcript increase at a single RQ-PCR assessment: 29 pts had less than 1-log increase and 41 pts had a 1-log increase or more – but with no loss of MMR. None of these pts was found to have mutations. Another 36 pts had less than 1-log increase and 53 had a 1-log increase or more, leading to loss of MMR. Mutations were detected in 1 (2.8%) and 3 (5.7%) pts, respectively. In pts receiving dasatinib or nilotinib as 2nd-line agents, mutation analysis was recommended at baseline and then in case of failure according to the provisional definitions proposed by Baccarani et al (J Clin Oncol 2009). Nineteen among the pts we analyzed met these criteria; overall, mutations were detected in 11 (57.8%), including 5/7 pts with no CyR at 3 months, 6/9 pts with minimal CyR at 6 months, 1/4 pts with less than PCyR at 12 months. In addition, newly acquired mutations were detected in 93/131 (71%) pts who lost a previously achieved HR or CyR. We also tested 19 pts who met the provisional definitions for suboptimal response to dasatinib or nilotinib 2nd-line. Mutations were detected in 4/19 pts (21%), including 2/5 pts with minor CyR at 3 months, 1/7 pts with PCyR at 6 months, 1/7 pts with less than MMR at 12 months. Our data indicate that: a) pts harbouring mutations can more frequently be found among cytogenetic suboptimal responders than among molecular suboptimal responders; b) any Bcr-Abl transcript increase that is not associated with MMR loss shouldn't indeed trigger a mutation analysis; c) although definitions of response to dasatinib or nilotinib 2nd-line are still provisional and might soon be refined, not only failures but also suboptimal responses are frequently associated with mutations.

58 % of failures
21% of suboptimals

More frequent amongst cyto rather than molec suboptimals

2.5. **[113] Imatinib Dose Interruption in Responding CML Patients Is Associated with Characteristic BCR-ABL Kinetics, Which Could Help to Differentiate Non-Adherence From Drug Resistance.** *Branford.* Introduction. Adherence to therapy is known to be a critical factor for achieving an optimal imatinib (IM) response, and non-adherence is likely to be a cause of loss of response. In the absence of a biological marker of resistance, such as BCR-ABL mutations or blast crisis (BC), it is difficult to determine whether loss of response is associated with non-adherence or drug resistance. Prior studies found that the rate of a BCR-ABL rise provided biological insight into the disease phase at relapse (Blood 1996 87 4473;

Blood 2004 104 2926). A more rapid rate of rise occurred in patients (pts) who relapsed into advanced phases compared to those who relapsed into chronic phase (CP), as assessed by the number of days over which BCR-ABL doubled (doubling-time, DT). We compared the DTs of pts with progression to BC and those who acquired a BCR-ABL mutation and maintained CP to the DTs of responding pts with documented IM discontinuation/interruption. The aim was to determine whether IM interruption was associated with characteristic BCR-ABL kinetics and loss of response, and whether the DT could help to differentiate drug resistance from non-adherence. Method*.* The molecular data was examined for 179 CP pts who achieved a major cytogenetic response and met these inclusion criteria: relapsed directly into BC at the time of a significant BCR-ABL rise; or a mutation was detected and CP was maintained at the time of a rise; or discontinued IM while in a sustained complete molecular response (CMR) as part of a discontinuation study; and/or IM dose was documented for BCR-ABL measurement intervals. IM dose interruption was defined as zero IM for ≥10% of days of a BCR-ABL measurement interval. Results*.* Twelve pts relapsed directly into BC and their BCR-ABL DT was short (median 9.0 days (d), range (r) 6.1-17.6); 8 had mutations and there was no DT difference for these pts. In contrast, 28 pts who maintained CP after mutation detection had a longer DT: median 45 d (r 17-140), *P*<.0001 (Figure). These pts met one or more criteria for IM failure (only 1 subsequently progressed to BC on IM after 8 months). While in CMR, 36 pts discontinued IM and 17 had a significant BCR-ABL rise and molecular relapse. The DT (median 9.0 d, r 6.9-27) was similar to the BC pts, *P*=.52. The short DT of pts who discontinued IM is consistent with the rate at which terminally differentiated leukemic cells arise from leukemic progenitors in the absence of IM (Nature 2005 435 1267, 8 d DT). The similarly short DT of BC pts suggests complete lack of BCR-ABL inhibition by IM, whereas relapse into CP with mutations was characterized by slow BCR-ABL kinetics, presumably due to maintenance of partial BCR-ABL inhibition by IM. We determined whether temporary IM dose interruption in responding pts also resulted in a BCR-ABL rise and characteristic DTs. IM interruption occurred in 42 pts (46 interruptions) for: intolerance 32; non-adherence 8; second malignancy/surgery 3; other 3. The IM interruptions were divided into 2 groups according to the days of zero IM: 100% of BCR-ABL measurement interval days (complete lack of BCR-ABL inhibition); or 10 to <100% of days (partial BCR-ABL inhibition). Twelve pts had 100% IM interruption and all had a rapid BCR-ABL rise and short DTs (median 9.4 d, r 4.2-17.6), consistent with the kinetic pattern of BC and discontinued in CMR pts, *P*=.78. Loss of response occurred in 11/12. Thirty pts had 34 measurement intervals of zero IM for 10 to <100% of days (median 33%, r 10-89). A BCR-ABL rise occurred in 27/34 interruptions and the DT (median 26 d, r 11.6-87) was significantly longer than BC, discontinued in CMR and 100% interruption pts, *P*<.0001. BCR-ABL remained stable in 5/34 interruptions and a reduction occurred in 2. Loss of response occurred in 18/34 interruptions. Of all 46 measurement intervals with an IM interruption (complete or partial), a significant BCR-ABL rise occurred in 39 (85%) and loss of response in 29 (63%), including 7/8 interruptions due to non-adherence. No pt progressed during the interruption. Twenty-one pts with a mutation and/or BC relapse had dose data available: none had an IM interruption during the relevant measurement interval. Conclusion*.* IM interruption is associated with a BCR-ABL rise. Intermittent IM dosing may be associated with a longer DT, similar to that seen with the emergence of mutations in CP. A short BCR-ABL DT is typical of complete loss of BCR-ABL inhibition and, for a patient still in CP, should raise the suspicion of non-adherence.

1.1.

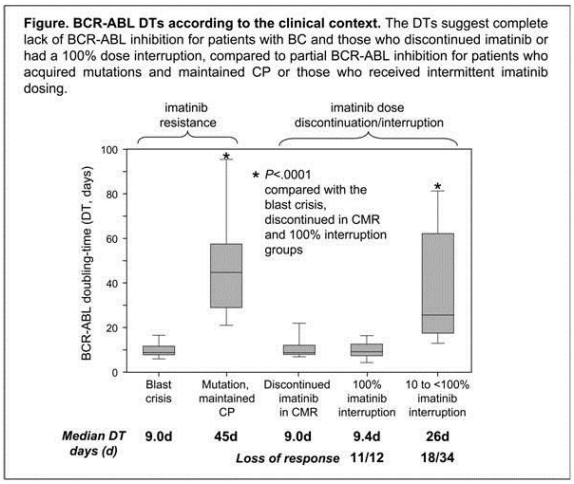


Figure. BCR-ABL DTs according to the clinical context. The DTs suggest complete lack of BCR-ABL inhibition for patients with BC and those who discontinued imatinib or had a 100% dose interruption, compared to partial BCR-ABL inhibition for patients who acquired mutations and maintained CP or those who received intermittent imatinib dosing.

2.6. **[114] Results From the ENESTnd Extension Study: Efficacy and Safety of Patients (pts) with Chronic Myeloid Leukemia in Chronic Phase (CML-CP), Treated with Nilotinib 400 Mg Twice Daily (BID) After Suboptimal Response (SoR) or Treatment Failure (TF) to Imatinib 400 Mg Once Daily (QD) or Nilotinib 300 Mg BID.** *Hochhaus. Background:* In ENESTnd, nilotinib demonstrated superior efficacy vs imatinib in pts with newly diagnosed CML-CP, including a significantly reduced rate of progression to AP/BC on treatment and a lower rate of SoR/TF. Pts in ENESTnd with SoR/TF on nilotinib 300 mg BID or imatinib could discontinue core study and enter an extension study; entrance was not allowed for intolerance. Here, we report the efficacy and safety of 49 such pts. *Methods:* 31 pts initially randomized to imatinib 400 mg QD (IM group) and 18 pts to nilotinib 300 mg BID (NIL group) in ENESTnd discontinued due to SoR/TF and received nilotinib 400 mg BID in this study. Progression and deaths in the extension study and after discontinuation of extension treatment have been previously reported as progression events after discontinuation of core ENESTnd treatment and in the OS analysis of ENESTnd. *Results:* Median time on extension treatment was 6 months (mo) for both groups (range, IM 0.2-24; NIL 1-14); 35/49 pts (71%) remain on study. Median nilotinib dose during extension treatment was equal to planned dose (800 mg/day). In the IM group, 65% of pts escalated imatinib to 400 mg BID prior to extension; 12 pts (46%) not in CCyR at extension entry and 7 pts (23%) not in MMR achieved these responses on extension treatment (table). Of these responders, 7/12 pts (58%) who achieved CCyR and 4/7 pts (57%) who achieved MMR had escalated imatinib to 400 mg BID on core study. In the NIL group, 1 pt (17%) not in CCyR at extension entry and 5 pts (29%) not in MMR, achieved this response on extension treatment. Overall, 4 pts in the IM group progressed to AP/BC (2 on extension treatment, 1 within 1 mo and the

Imatinib 400 mg QD (IM) pts in ENESTnd who discontinued due to SoR/TF and entered extension (n = 31)	
Pts with imatinib dose escalation to 400 mg BID prior to extension, n (%)	20 (65)
Reason for entering extension, n (%)	
TF*	21 (68)
SoR†	8 (26)
Other#	2 (6)
Response achieved during extension, n (%)	
Response prior to entry < CCyR (n = 26)	12 (46)
Response prior to entry < MMR (n = 30)	7 (23)
Nilotinib 300 mg BID (NIL) pts in ENESTnd who discontinued due to SoR/TF and entered extension (n = 18)	
Reason for entering extension, n (%)	
TF*	3 (17)
SoR†	14 (78)
Other#	1 (6)
Response achieved during extension, n (%)	
Response prior to entry < CCyR (n = 6)	1 (17)
Response prior to entry < MMR (n = 17)	5 (29)

*TF: no CyR at 6 mo; < PCyR at 12 mo; < CCyR at 18 mo; loss of confirmed CHR, PCyR, CCyR, progression to AP/BC or clonal evolution at any time.
†SoR: < PCyR at 6 mo; < CCyR at 12 mo; < MMR at 18 mo.
#Entered extension per investigator assessment without satisfying criteria for SoR/TF.

other > 12 mo after discontinuation). All 4 pts discontinued core study for TF. Overall, 1 pt in the NIL group progressed to AP/BC (< 1 mo after discontinuation of extension treatment); pt discontinued core study for SoR. The safety of nilotinib 400 mg BID was similar to that in the core study. Grade 3/4 AEs and drug-related AEs leading to discontinuation were reported in 52% and 10% of pts in the IM group. Higher rates of AEs in the first few mo of starting nilotinib in pts previously treated with imatinib were not unexpected as common AEs occur early following initial exposure. In the NIL group, grade 3/4 AEs were reported in 28% of pts and no pt discontinued due to drug-related AEs. No deaths were reported on extension treatment or ≤ 28 days of discontinuation; 4 deaths occurred > 28 days after discontinuation of treatment: 3 were CML-related (2 and 1 deaths in the IM and NIL groups, respectively) and occurred 8-10 mo after discontinuation. *Conclusions:* Results confirm the efficacy of nilotinib 400 mg BID for pts with CML-CP who had SoR/TF on imatinib, even after imatinib dose escalation. These results suggest that nilotinib 400 mg BID may be efficacious in pts with CML-CP with SoR/TF on nilotinib 300 mg BID, although longer follow-up is required. Whereas dose escalation of imatinib may overcome OCT-1 transporter activity in pts with correspondingly low imatinib plasma levels, nilotinib is not a substrate for OCT-1. The modest (~16%) increase in nilotinib systemic exposure by dose escalation from 300 to 400 mg BID may benefit some patients with SoR/TF, but requires further evaluation. Currently, dose escalation of nilotinib from 300 to 400 mg BID appears safe with no additional safety signals. The extension study is ongoing and additional follow-up will provide more information moving forward.

3 Biology and Pathophysiology: Genetic Instability and Requirements for survival [445-450]

3.1. **[445] Genomic Instability Originates From Leukemia Stem Cells in a Mouse Model of CML-CP.** *Bolton.* For decades, chronic myeloid leukemia (CML) has served not only as a paradigm for understanding the evolution and multi-step process of carcinogenesis but also for studying cancer stem and progenitor cells responsible for the initiation and/or maintenance of the disease. CML is initiated by BCR-ABL1 tyrosine kinase transformation of hematopoietic stem cells into leukemia stem cells (LSCs) to induce CML-chronic phase (CML-CP). The deregulated growth of LSC-derived leukemia progenitor cells (LPCs) leads to manifestation of the disease. It is unclear if LSCs and/or LPCs are able to acquire additional genetic changes that confer resistance to tyrosine kinase inhibitors (TKIs) and induce more aggressive CML blast phase (CML-BP). In addition, the mechanisms and consequences of genomic instability may differ substantially among these cells. For example, the effects of genetic aberrations acquired in quiescent LSCs may be dormant, but if the aberrations induce proliferation or appear in LSCs that are already cycling, they may generate TKI-resistant and/or more malignant clones. Alternatively, genomic instability in LPCs must be accompanied by the acquisition of LSC-like properties to prevent mutations from disappearing before they undergo terminal maturation. Previously, we reported that BCR-ABL1–transformed cell lines accumulate reactive oxygen species (ROS)-induced oxidative DNA damage [8-oxoguanine (8oxoG), double strand breaks (DSBs)] resulting in genomic instability *in vitro*, which was responsible for acquired imatinib-resistance and accumulation of chromosomal aberrations (*Nowicki et al., Blood, 2005; Koptyra et al., Blood, 2006; Koptyra et al., Leukemia, 2008*). To determine which populations of CML-CP cells, LSCs and/or LPCs, accumulate genomic instability we employed the SCLtTA/BCR-ABL1 tetracycline-inducible (tet-off) transgenic mouse model of CML-CP with targeted expression of p210BCR-ABL1 in hematopoietic stem and progenitor cells (*Koschmieder et al., Blood, 2005*). Mice exhibiting CML-CP-like disease resulting from BCR-ABL1 induction demonstrated splenomegaly and $Gr1^+/CD11b^+$ myeloid expansion in bone marrow, spleen and peripheral blood. BCR-ABL1 mRNA expression was higher in the $Lin^-c\text{-}Kit^+Sca1^+$ murine leukemia stem cell–enriched population (muLSCs) than in the $Lin^-c\text{-}Kit^+Sca1^-$ murine leukemia progenitor cell–enriched population (muLPCs), thus reminiscent of human CML-CP ($Lin^-CD34^+CD38^-$ LSCs > $Lin^-CD34^+CD38^+$ LPCs). BCR-ABL1 induction increased levels of ROS (hydrogen peroxide, hydroxyl radical) and oxidative DNA damage (8-oxoG, DSBs) in muLSCs, but not in muLPCs. In addition, $CFSE^{max}$/$eFluor670^{max}$ quiescent muLSCs displayed more ROS (superoxide, hydrogen peroxide) and oxidative DNA damage (8oxoG, DSBs) than non-induced counterparts. Currently, we are examining genomic instability in the most primitive long-term muLSCs ($Lin^-c\text{-}Kit^+Sca1^+CD34^-Flt3^-$). Lastly, single nucleotide polymorphism (SNP) arrays detected a variety of genetic aberrations (addition, deletions) in BCR-ABL1–induced Lin^- BM cells. Individual mice displayed a great degree of diversity in the intensity of genetic instability accumulating between 31 to 826 aberrations, which recapitulate heterogeneity of sporadic aberrations detected in CML-CP patients. These aberrations include deletions in Trp53 and Ikzf1, and additions in Zfp423 and Idh1 genes, which have been linked to progression from CML-CP to CML-BP. In summary, by using the SCLtTA/BCR-ABL1 inducible transgenic mouse model of CML-CP we showed that muLSCs, but not muLPCs, displayed elevated levels of ROS-induced oxidative DNA damage likely resulting in the accumulation of extensive genetic aberrations. This observation supports the hypothesis that genomic instability in CML-CP originates in LSCs. Current analysis of microarrays may shed some light on the mechanisms leading to enhanced ROS production and accumulation of oxidative DNA damage in muLSCs.

3.2. **[446] BCL6-Mediated Repression of p53 Is Critical for Leukemia Stem Cell Survival in Chronic MyeloidLeukemia .** *Hurtz.* **Background:** Chronic myeloid leukemia (CML) is induced by the oncogenic *BCR-ABL1* tyrosine

kinase and can be effectively treated for many years with tyrosine kinase inhibitors (TKI). However, unless CML patients take TKI-treatment life-long, leukemia will eventually recur, which is attributed to the failure of TKI-treatment to eradicate leukemia-initiating cells (LIC; Corbin et al., J Clin Invest 2011). Persistence of LIC in CML can result in acquisition of secondary events eventually leading to TKI-resistant blast crisis, which is fatal within months. Recent work demonstrated that FoxO factors are critical for maintenance of CML-initiating cells (Naka et al., Nature 2010), however the mechanism of FoxO-dependent leukemia-initiation remained elusive. **Results:** Here we identified the BCL6 protooncogene as a critical effector downstream of FoxO in self-renewal signaling of CML-initiating cells. ChIP-seq analysis demonstrated that BCL6 directly binds to and represses Arf and p53 promoters in human CML cells. Genetic deletion of the BCL6 gene in a mouse model of CML results in progressive depletion of Lin- Sca-1+ c-Kit+ LIC. BCL6-deficient LIC exhibit excessively high expression levels of Arf and p53 and propensity to cellular senescence and apoptosis. As a consequence, BCL-deficient CML cells lack the ability to form colonies and to initiate leukemia in transplant recipient animals. To investigate whether these effects are indeed owing to the role of BCL6 as repressor of Arf/p53, we induced activation of a dominant-negative BCL6-mutant in p53+/+ and p53-/- CML cells. While dominant-negative BCL6 compromised colony formation and self-renewal in p53+/+ CML cells, BCL6 inhibition only had minor effect on p53-/- CML cells. We conclude that BCL6 enables survival of LIC in CML mainly through transcriptional repression of p53. To test potential clinical relevance of these findings, we used a recently developed retro-inverso BCL6 peptide inhibitor (RI-BPI, Cerchietti et al., 2009), which inhibits BCL6 function as transcriptional repressor. RI-BPI is currently under clinical trial for the treatment of BCL6-dependent diffuse large B cell lymphoma (Dr. Ari Melnick, LLS TAP Program). Importantly, peptide inhibition of BCL6 in human CML cells compromises colony formation and leukemia-initiation in transplant recipients and selectively eradicates $CD34^+$ $CD38^-$ LIC in patient-derived CML samples. **Conclusions:** These findings identify pharmacological inhibition of BCL6 as a novel strategy to eradicate LIC in CML. Clinical validation of this concept could limit the duration of TKI-treatment in CML patients, which is currently life-long, and substantially decrease the risk of blast crisis transformation. Based on these findings, we propose a dual targeting strategy, in which (1) tyrosine kinase inhibitors (e.g. Imatinib) to target the transient amplifying pool of CML cells are coupled with (2) BCL6 inhibition that will target quiescent LIC.

3.3. **[447] Targeting DNA Repair Gene, RAD52, Induces Exhaustion of the Proliferating CML-CP Leukemia Stem Cells Carrying Oxidative DNA Damage.** *Cramer.* BCR-ABL1 transforms hematopoietic stem cells (HSCs) into leukemia stem cells (LSCs) to induce chronic myeloid leukemia in chronic phase (CML-CP). We detected that the most primitive LSCs display elevated levels of reactive oxygen species (ROS) and accumulate excessive numbers of potentially lethal DNA double-strand breaks (DSBs). We also reported that BCR-ABL1-transformed cells exhibit enhanced RAD51-mediated homologous recombination repair (HRR) activity occurring in S and G2/M cell cycle phases. In normal cells initiation of RAD51-mediated HRR is directed either by BRCA1– or RAD52–dependent mechanisms. Since BCR-ABL1 kinase downregulated BRCA1, LSCs containing high number of DSBs should depend more on RAD52 to promote HRR to repair lethal DSBs. We found that in vivo leukemogenic potential of BCR-ABL1 –positive RAD52-/- hematopoietic cells is abrogated in comparison to their BCR-ABL1 -positive RAD52+/+ counterparts. The absence of RAD52 in BCR-ABL1 –positive cells reduced the percentage of $Lin^-Kit^+Sca1^+$ cells by >2-fold and inhibited their clonogenic potential and proliferation by >10-fold. In addition RAD52 knockout caused approximately 2-fold reduction of $Lin^-Kit^+Sca1^+CD34^-Flt3^-$ long-term LSCs (LT-LSCs) and $Lin^-Kit^+Sca1^+CD34^+Flt3^-$ short-term LSCs (ST-LSCs). Conversely, 4-fold accumulation of BCR-ABL1 –positive RAD52-/- $Lin^-Kit^+Sca1^+eFluor670^{max}$ quiescent cells was detected in comparison to BCR-ABL1 –positive RAD52+/+ counterparts. These effects were accompanied by 2-fold reduction of the percentage of BCR-ABL1 –positive RAD52-/- cells in S and G2/M and 7-fold increase of these cells in sub-G1 when compared to BCR-ABL1 –positive RAD52+/+ counterparts. BCR-ABL1-positive RAD52-/- $Lin^-Kit^+Sca1^+$ cells accumulated more DSBs than BCR-ABL1 –positive RAD52+/+ cells. These differences were not observed between non-transformed RAD52-/- and RAD52+/+ cells. Expression of the wild-type RAD52 reduced the accumulation of lethal DSBs and rescued the clonogenic potential and proliferation of BCR-ABL1-positive RAD52-/- $Lin^-Kit^+Sca1^+$ cells. Downregulation of ROS with antioxidants vitamin E (VE) and N-acetyl-cysteine (NAC) exerted similar effect as restored expression of RAD52. Thus it appears that RAD52 is necessary to repair the extensive ROS-induced DSBs in LSC-enriched $Lin^-Kit^+Sca1^+$ cells. BCR-ABL1 kinase does not affect the expression of RAD52 protein, but phosphorylates RAD52 on Y104. However, expression of RAD52(Y104F) phosphorylation-less mutant reduced the number of DSBs and rescued the clonogenic potential of BCR-ABL1-positive RAD52-/- $Lin^-Kit^+Sca1^+$ cells in a similar way to the wild-type RAD52. Accordingly, RAD52-mediated DSB repair activity in CML-CP cells should not be affected by imatinib treatment. RAD52 mediates the annealing of complementarry DNA strands during DSB repair. To exert this function RAD52 has two DNA binding domains. Expression of RAD52(F79A) and RAD52(K102A) DNA binding-deficient mutants (each amino acid substitution inactivated different DNA binding domain) failed to prevent the accumulation of DSBs and did not rescue the clonogenic and proliferative potential of BCR-ABL1-positive RAD52-/- cells. In addition, RAD52(F79A), but not RAD52(Y104F) inhibited DSB repair by HRR. Therefore DNA binding capability of RAD52 appears essential for BCR-ABL1 –mediated leukemogenesis, but it is dispensable in normal hematopoietic cells. The “addiction” of BCR-ABL1 leukemia cells to RAD52 was confirmed by demonstration that RAD52(F79A) mutant inhibited clonogenic potential of CD34+ CML-CP cells, but not normal counterparts. Furthermore, to determine if RAD52 DNA binding domains could be targeted to selectively inhibit CML-CP, peptide aptamers containing RAD52 DNA binding domain amino acids sequence surrounding F79 were employed as potential decoys for RAD52 DNA binding. Aptamer containing F79, but not the A79 substitution, diminished the number of RAD52 foci and reduced the clonogenic potential and proliferation of $CD34^+$ cells from CML-CP, but not from normal donors. In conclusion, we postulate that RAD52 is essential for BCR-ABL1 –mediated leukemogenesis and that DNA binding domains of RAD52 may be targeted for selective elimination of the proliferating CML-CP LSCs.

3.4. **[448] Pharmacological Inhibition of the Stress-Related Deacetylase SIRT1 Enhances Eradication of CML stem Cells.** *Li.* BCR-ABL tyrosine kinase inhibitors (TKI) are effective in inducing remissions and prolonging survival of CML patients, but fail to eradicate primitive leukemia stem cells (LSC) which remain a potential source of relapse. New strategies

to enhance elimination of residual CML LSC in TKI-treated patients are required. We have previously reported that the stress-related deacetylase SIRT1 is expressed at high levels in CML stem/progenitor cells and that inhibition of SIRT1 expression using lentivirus-expressed shRNA induces apoptosis in CML progenitors and increases their sensitivity to imatinib (IM) by activating p53 signaling (Blood 2010, 116: 200A). These results support an important role for SIRT1 in CML LSC maintenance and TKI resistance, and as a potential molecular target for therapy directed against CML LSC. Tenovin-6 (TV) has been identified as a potent small molecule inhibitor of SIRT1 activity (Cancer Cell 2008, 13:454). Here we evaluated whether pharmacological inhibition of SIRT1 activity using TV could selectively inhibit CML stem/progenitor cells. As with shRNA-mediated knock-down of SIRT1, treatment with TV (0.5 μM) significantly increased apoptosis of CML CD34+ cells (TV16±7% vs. Control 3±2%, p=0.04, n=3), but not normal CD34+ cells (TV 6±2% vs. Control 4±2%, p=0.1, n=3). The combination of IM (2.5 μM) and TV induced significantly increased apoptosis in CML progenitors compared to IM alone, and to a significantly greater extent than in normal cells (CML, TV + IM 40±2% vs. IM 19±3%, p=0.009, n=3; CB, TV + IM 15±4% vs. IM 10±2%, p=0.04, n=3). TV (1 μM) increased apoptosis in both CML CD34+CD38- (TV 42±10% vs. Control 4±3%, p=0.04, n=3) and CD34+CD38+ cells (TV 35±7% vs. Control 8±2%, p=0.03, n=3). CFSE labeling indicated that treatment with TV resulted in increased apoptosis of undivided CML CD34+CD38- cells identified on the basis of high CFSE fluorescence (TV 20±7% vs. Control 2±1%, p=0.04, n=3). The combination of TV with IM resulted in a significant increase in apoptosis in CML CD34+CD38- $CFSE^{high}$ cells compared to IM alone (TV plus IM 35±5% vs. IM 10±4%, p=0.03, n=3). Treatment with TV (0.5 μM) reduced CML CFC frequency (70±9% inhibition with TV compared to untreated controls, p=0.009, n=3) without affecting normal CFC frequency. Combination of TV (0.5 μM) with IM resulted in enhanced inhibition of CML CFC compared to IM alone, but did not enhance inhibition of normal CFC (CML: TV plus IM 82±6% inhibition vs. IM 57±10%, p=0.02, n=3; CB: TV plus IM 38±7% inhibition vs. IM 36±9%, p=0.1, n=3). TV treatment effectively inhibited the growth of Baf3 cells expressing T315I-mutated BCR-ABL, and significantly enhanced apoptosis of IM-resistant CML blast crisis CD34+ cells [TV (1 μm) 30±1% vs. Control 19±5%, p=0.04, n=3], suggesting SIRT1 inhibition can also target TKI-resistant CML cells. Ex vivo treatment with TV (1 μM) significantly reduced longer-term (12 weeks) engraftment of CML CD34+ cells in NSG mice following TV treatment (TV treated $0.2*10^5 \pm 0.1*10^5$ human CD45+ cells in murine BM vs. Control $1.8*10^5 \pm 0.6*10^5$, p=0.009, n=5). Significant reduction in engraftment of CD33+ (p=0.008) and CD14+ myeloid cells (p=0.009) was seen. Q-PCR and FISH analysis confirmed that engrafted human cells were leukemic in origin. Interestingly, engraftment of CB CD34+ cells was not reduced after treatment with TV (TV $2.7*10^6 \pm 0.7*10^6$ human CD45+ cells in murine BM, vs. Control $2.4*10^6 \pm 0.8*10^6$, p=0.2, n=6). These results show that SIRT1 inhibition by TV effectively targets primitive human CML cells with in vivo multi-lineage engraftment capacity. Treatment with TV significantly enhanced acetylated p53 levels in CML CD34+ cells, indicating effective inhibition of SIRT1 activity. TV treatment also increased total p53 levels, possibly related to reduced p53 degradation. TV treatment did not increase acetylated p53 or total p53 levels in normal CD34+ cells. Importantly shRNA-mediated knock-down of p53 resulted in significant reduction of TV-induced apoptosis in CML CD34+ cells (13±6% apoptosis with p53 shRNA; 33±7% apoptosis with control shRNA, p=0.04, n=3), indicating that the effects of TV on CML CD34+ cells are related to p53 acetylation and activation. In conclusion, our studies indicate that pharmacological inhibition of SIRT1 can activate p53 and enhance eradication of CML LSC in combination with TKI treatment, and support further evaluation of targeted inhibition of SIRT1 as a therapeutic strategy in CML.

3.5. [449] *HIF1α* Is Required for Survival Maintenance of Chronic Myeloid Leukemia Stem Cells. *Zhang.* Chronic myeloid leukemia (CML) is a clonal hematopoietic stem cell disorder induced by the BCR-ABL oncogene, and available BCR-ABL kinase inhibitors fail to completely eradicate leukemia stem cells (LSCs) to cure the disease. The challenge lies in the identification of genes that play a critical role in survival regulation of LSCs. Hypoxia-inducible factor-1α (*HIF1α*), a master transcriptional regulator of the cellular and systemic hypoxia response, is essential for the maintenance of self-renewal capacity of normal hematopoietic stem cells (HSCs). It is still unknown about the role of *HIF1α* in survival regulation of LSCs in CML. Using a mouse model of CML, here we report that *HIF1α* plays a crucial role in survival maintenance of LSCs. We conducted a DNA microarray analysis to compare the gene expression profiles between LSCs and normal HSCs in our bone marrow transplantation (BMT) mouse model of CML. We retrovirally transduced bone marrow cells from C57BL/6J (B6) mice with BCR-ABL-GFP or GFP alone (as a normal HSC control) and transplanted the transduced cells into lethally irradiate B6 recipient mice to induce CML. Two weeks after BMT, we sorted GFP^+LSK ($Lin^-Sca\text{-}1^+c\text{-}Kit^+$) cells from bone marrow of the mice for the Affymetrix microarray analysis. *HIF1α* gene was up-regulated by BCR-ABL in LSCs. We next examined expression of genes known to be specifically regulated by *HIF1α*, and found that expression of *VEGF*, *GLUT1* and *TGFa*, except for PGK1, were significantly higher in LSCs than in HSCs. Real time RT-PCR assay confirmed the up-regulation of *HIF1a* and other hypoxia-responsive genes by BCR-ABL in LSCs. To determine the role of HIF1α in BCR-ABL leukemiogenesis, we crossed mice carrying a *loxP*-flanked *HIF1a* allele with *Cre* transgenic mice in which expression of *Cre* is driven by the *Vav* regulatory element to induce the deletion of the HIF1a gene mainly in the hematopoietic system. We transduced bone marrow cells from 5-FU-treated wild type (WT) or *HIF1a*$^{-/-}$ mice with BCR-ABL-GFP retrovirus, and then transplanted into lethally irradiated recipient mice to induce primary CML, followed by a secondary transplantation. We found that *HIF1α*$^{-/-}$ LSCs failed to induce CML in the secondary recipient mice, whereas WT LSCs efficiently induced CML. The defective CML phenotype in the absence of *HIF1α* was consistent with a gradual decrease of the percentages and total numbers of leukemia cells in peripheral blood and with much less severe splenomegaly. These results indicate that *HIF1α* is required for CML development, and suggest that HIF1α is required for survival maintenance of LSCs. To understand the underlying mechanisms, we analyzed the effect of *HIF1α* on cell cycle progression and apoptosis of LSCs, and found that the percentage of *HIF1α*$^{-/-}$ LSCs in the S-G2/M phase was significantly lower than that of WT LSCs, indicating that the *HIF1α* deficiency causes a cell cycle arrest of LSCs. Furthermore, we examined whether deletion of *HIF1α* induces apoptosis of LSCs by staining the cells with annexin V and 7AAD, and found that *HIF1α*$^{-/-}$ LSCs had a higher apoptotic rate than WT LSCs. We further compared expression levels of three cyclin-dependent kinase inhibitors p16Ink4a, p19Arf, and p57 between *HIF1α*$^{-/-}$ and WT LSCs, and found that the cell cycle arrest caused by the *HIF1α* deficiency was associated with significantly higher levels of expression of p16Ink4a, p19Arf and p57 in *HIF1α*$^{-/-}$ LSCs than in WT LSCs. In addition, we observed an increased expression of the apoptotic gene p53 in *HIF1α*$^{-/-}$ LSCs, explaining the increased

apoptosis of *HIF1α*$^{-/-}$ LSCs. In summary, our results demonstrate that *HIF1α* represents a critical pathway in LSCs and inhibition of the *HIF1α* pathway provides a therapeutic strategy for eradicating LSCs in CML.

3.6. **[450] Physiologic Hypoxia Promotes Maintenance of CML Stem Cells Despite Effective BCR-ABL Inhibition.** *Ng.* Chronic myelogenous leukemia (CML) is a disease of hematologic stem cells caused by the BCR-ABL kinase. The use of the ABL kinase inhibitor, imatinib (IM), has dramatically improved the control of CML, but does not cure it. Current evidence suggests that CML stem cells are able to survive IM treatment, and initiate disease recurrence when IM therapy is stopped. The eradication of residual CML stem cells will depend on the identification of mechanisms that support their persistence. The bone marrow microenvironment supports the growth and renewal of hematopoietic stem cells (HSC), and possibly, CML stem cells. Recent studies reveal that physiologic hypoxia (0.1-10%) is a critical component of the microenvironment. We hypothesize that hypoxia maintains CML stem cell function, and that it can do so despite effective BCR-ABL kinase inhibition. To test this hypothesis, we characterized the effect of hypoxia on immunophenotypically-defined CML progenitor and stem cells in the presence and absence of IM. To examine the effect of hypoxia on CML progenitors, we first performed colony forming assays (CFA) on primary chronic phase (CP) CML cells treated with IM (0, 0.25, 1 and 5 μM) under hypoxic (0.5 O_2) or normoxic (21% O_2) conditions for 96 hours. We found that hypoxia increased the number of colonies by 1.6- and 3.2- fold compared to 21% O_2 at 0 and 5μM IM respectively, suggesting that hypoxia favors the maintenance of CML progenitors. To explore the relationship between cell proliferation and hypoxia, we employed CFSE staining to track cell divisions. We found that while both $CD34^+CD38^+$ (committed progenitors) and $CD34^+CD38^-$ (stem cells) CP CML cells proliferated more slowly under hypoxia alone (~20% decrease in the proliferation index), hypoxia did not further enhance the anti-proliferative effects of IM in both populations. Together, these observations suggest that hypoxia enhances the clonogenic activity of CML progenitors, and that it is able to do so despite the anti-proliferative effects of IM. To rule out the possibility that hypoxia impaired the ability of IM to inhibit BCR-ABL kinase, we also examined the level of phospho-CrkL by intracellular flow cytometry in the $CD34^+CD38^+$ and $CD34^+CD38^-$ CML populations. We found that IM reduced the level of pCrkL in both $CD34^+CD38^+$ and $CD34^+CD38^-$ CML cells, and that hypoxia had no effect on the degree of pCrkL reduction. In line with this result, we also found that hypoxia had no effect on the degree of apoptosis induced by IM. These results indicated that hypoxia increased the clonogenic activity of CML progenitors, and that this occurred in a BCR-ABL-independent manner. These data encouraged us to determine if hypoxia had similar effects on the CML stem cell, which we assayed by performing limiting dilution LTCIC assays, and by assessing the ability of hypoxia-exposed CML cells to engraft immunodeficient NSG mice. In both assays, CML cells incubated under hypoxia showed increases in stem cell capacity (Figure 1). Taken together, our results indicate that physiologic hypoxia maintains the ability of CML cells to act as leukemia stem cells (LSC), and that this can occur in a BCR-ABL-independent manner. In this respect, the functional effects of hypoxia on CML stem cells mirror those on normal HSCs (Danet et al. JCI, 2003). To provide support for this notion, we performed gene expression analysis using Affymetrix U133 Plus 2.0 chips on $CD34^+$ cells obtained from normal individuals, as well as CML patients in CP and blast crisis (BC). Preliminary analyses of the microarray data suggest that the hypoxic response in normal and $CD34^+$ CP cells was remarkably similar, in contrast to the $CD34^+$ BC response. Together, our data indicate that: 1. Physiologic hypoxia enhances the capacity of CP CML progenitors to function as LSCs; 2. Hypoxia enhances CP LSC function independently of BCR-ABL; 3. CP $CD34^+$ CML cells have a similar gene expression response to hypoxia compared to normal $CD34^+$ cells. Our findings suggest that therapies targeting BCR-ABL will not eliminate CP CML stem cells, particularly in the context of a hypoxic microenvironment.

4 Therapy: New First Line Options [451-456]

4.1. **[451] Upfront Imatinib Therapy in CML Patients with Rapid Switching to Nilotinib for Failure to Achieve Molecular Targets or Intolerance Achieves High Overall Rates of Molecular Response and a Low Risk of Progression - An Update of the TIDEL-II Trial.** *Yeung.* Background: While nilotinib and dasatinib produce faster responses than imatinib as first-line therapy in *de novo* Chronic Phase Chronic Myeloid Leukemia (CP-CML), an equally effective strategy may be to selectively use these more potent tyrosine kinase inhibitors (TKIs) only in patients who fail to achieve stringent early molecular targets or are intolerant. Aim: To update the molecular outcome and survival of patients in the TIDEL-II study. Method: TIDEL-II is a multicentre, single arm prospective ALLG trial for de novo CP-CML adult patients with two sequential cohorts each of 105 patients. All patients started on imatinib (IM) 600mg OD. Patients with IM trough levels <1000ng/mL on day 22 were dose escalated to 800mg OD (IM800). All patients were monitored for achievement of time-dependent molecular targets - BCR-ABL RQ-PCR of 10%, 1% and 0.1% IS at 3, 6 and 12 months (mo) respectively. Patients in cohort I who failed to meet these targets had dose escalation to IM800. Those patients who again failed to achieve these targets after a further 3 mo were switched to nilotinib 400mg BID (NIL). Patients in cohort 2 who failed their time dependent targets switched to NIL directly without escalating to IM800. In both cohorts, switching to NIL was also permitted for grade III/IV or persistent grade II non-haematological toxicity or loss of response. Primary end point was MMR at 12 mo (BCR-ABL ≤0.1%IS), with $CMR^{4.5}$ being a secondary end point (BCR-ABL ≤0.0032%IS). Results: At 12 mo 69% of patients achieved MMR. With median follow up (f/u) of 20mo, AP/BC progression occurred in 5 cases (2.4%) (table 1). The 3 mo molecular response was highly correlated with the MMR at 12mo and progression events (table 2). COHORT 1: Using intention to treat analysis (ITT) with median follow-up of 30 mo the rate of MMR at 12 and 24 mo is 66% and 81% respectively (n=105); $CMR^{4.5}$ was 12% and 24%, respectively. In total, 34/105 (32%) patients switched to NIL, 12 for failure to achieve molecular targets, 19 for intolerance and 3 for loss of response. Only 2/12 patients who failed to meet targets on IM have subsequently achieved MMR on NIL (median f/u on NIL 14 mo). Fourteen patients switched for intolerance when not in MMR, and 9 subsequently gained MMR (64%) (median f/u on NIL 19 mo). Two patients progressed to AP/BC, both in the first 12 mo in patients taking IM. One progression related death and one fatal myocardial infarction (on NIL) have been reported. Fourteen (13%) of patients remain on IM800. COHORT 2: With a median f/u of 12 mo the rates of MMR and $CMR^{4.5}$ at 12 mo (n=50) were 72% and 16%, respectively (ITT). To date, 35/105 patients, (33%) have switched to NIL, of which 23 switched for failure to meet molecular targets. Subsequently, 3/23 (13%) have achieved MMR (median 6 mo on

NIL). Eleven patients have switched to NIL for intolerance, 7 of them not in MMR at time of switch; 6/7 reached MMR in the subsequent 6 mo (median 5 mo on NIL). Seven patients (7%) remain on IM800. Three patients progressed to AP/BC (3%), 2 on IM and 1 on NIL. Three deaths were reported (3%), 1 from cardiac causes and 1 from stroke, both patients on IM at the time; and 1 from CML progression. Relatively short f/u precludes a meaningful comparison of results between the 2 cohorts. Conclusion: The TIDEL-II strategy has achieved a higher rate of MMR at 12 mo of 69% compared to 47% achieved with the strategy of IM intensification previously utilised in the TIDEL-I study. The improvement in molecular response is mostly attributable to improved responses in patients intolerant of IM as deeper responses were uncommon with patients who failed their early molecular targets despite intensification of kinase inhibition. Molecular response at 3 mo is highly correlated with response and progression events, underscoring the importance of early molecular targets.

Table 1. Patient characteristics and summary results

	Cohort 1	Cohort 2	All patients
n	105	105	210
Follow up in mo (median)	20-45 (30)	4-20 (12)	4-45 (20)
MMR at 12 mo	66%	72%	69%
$CMR^{4.5}$ at 12 mo	12%	16%	14%
Switched to NIL by 12 mo	17%	33%	25%
Transformation to BC	2 (1.9%)	3 (2.9%)	5 (2.4%)
Death	2 (1.9%)	3 (2.9%)	5 (2.4%)
Total Withdrawal	17 (16.2%)	9 (8.6%)	26 (12.3%)

Table 2. Response according to BCR-ABL%IS at 3 months

Median follow up 20 months

BCR-ABL%IS at 3 months		MMR @ 12 months	Progression to BC
<1	64%	87%	1%
1 - <10	26%	38%	5%
10	10%	27%	13%
p value (Fisher's)		<0.001	0.025

4.2. **[452] Nilotinib Versus Imatinib in Patients (pts) with Newly Diagnosed Philadelphia Chromosome-Positive (Ph+) Chronic Myeloid Leukemia in Chronic Phase (CML-CP): ENESTnd 36-Month (mo) Follow-up.** *Saglio. Background:* In ENESTnd, pts treated with nilotinib demonstrated higher and faster rates of major molecular response (MMR, ≤ 0.1% $BCR\text{-}ABL^{IS}$), deeper molecular response (MR^4, ≤ 0.01%^{IS} and $MR^{4.5}$, ≤ 0.0032%^{IS}), and complete cytogenetic responses (CCyR) along with significantly lower rates of progression to AP/BC and fewer CML-related deaths compared with imatinib by 12 and 24 mo. Here, we report data with a minimum follow-up of 24 mo; however, efficacy and safety data based on considerably longer follow-up of ≥ 36 mo will be presented. As demonstrated in IRIS and other imatinib trials, most pts who progress on imatinib do so within the first 3 years of therapy. Thus, this 36-mo update of ENESTnd will be important to further verify the benefits of nilotinib in newly-diagnosed pts. *Methods:* 846 adult pts with newly-diagnosed Ph+ CML-CP were randomized to nilotinib 300 mg twice daily (BID) (n = 282), nilotinib 400 mg BID (n = 281), or imatinib 400 mg once daily (QD) (n = 283). MMR, MR^4, $MR^{4.5}$, time to progression to AP/BC on treatment, progression-free survival (PFS) on treatment, and overall survival (OS) were evaluated. *Results:* By 24 mo, both doses of nilotinib demonstrated significantly

higher rates of MMR, MR^4, and $MR^{4.5}$ vs imatinib (Table). Nilotinib-treated pts achieved median $BCR\text{-}ABL^{IS}$ levels of 0.09% (300 mg BID) and 0.10% (400 mg BID) by 12 mo, while this level of reduction was not observed before 24 mo on imatinib. More pts with CCyR achieved MMR at 12 and 24 mo with either dose of nilotinib vs imatinib (Table). Regardless of Sokal risk, rates of MMR and $MR^{4.5}$ were higher for nilotinib at both doses vs imatinib (Table). Progression to AP/BC (excluding clonal evolution [CE]) on treatment was significantly lower for nilotinib vs imatinib (2 pts and 3 pts with nilotinib 300 mg BID [*P* = .0059] and 400 mg BID [*P* = .0196]), respectively vs 12 pts with imatinib). After achieving CCyR, 4 pts treated with imatinib progressed to AP/BC and 2 pts treated with nilotinib 400 mg BID progressed after achieving both CCyR and MMR (1 also achieved MR^4). No pt who achieved $MR^{4.5}$ progressed at any time. All but 1 pt who progressed to AP/BC on treatment were in the intermediate and high Sokal risk groups; 1 pt treated with nilotinib 400 mg BID progressed in the low Sokal risk group who had an E255V mutation at progression. When considering progression events of pts after discontinuation of treatment, an additional 7, 2, and 6 events (excluding CE) were observed with nilotinib 300 mg BID, nilotinib 400 mg BID and imatinib, respectively. Twice as many pts had emergent mutations on imatinib (n = 20) vs nilotinib (n = 10 on 300 mg BID; n = 8 on 400 mg BID). At 24 mo, OS remained similar in all groups, but there were fewer CML-related deaths in both nilotinib 300 mg BID (5 pts) and nilotinib 400 mg BID (3 pts) arms vs imatinib (10 pts). Both drugs were well tolerated and few new adverse events (AEs) and lab abnormalities were observed between 12- and 24-mo of follow-up. Nilotinib 300 mg BID had the fewest discontinuations due to AEs/lab abnormalities (9% vs 13% and 10% with nilotinib 400 mg BID and imatinib, respectively). *Conclusions:* With a minimum follow-up of 24 mo, nilotinib continued to demonstrate superiority vs imatinib with faster and deeper molecular responses and a significantly decreased risk of progression. These data support the use of nilotinib as a standard of care option in newly-diagnosed adult pts with Ph+ CML-CP.

	Nilotinib 300 mg BID (n = 282)	Nilotinib 400 mg BID (n = 281)	Imatinib 400 mg QD (n = 283)
MMR by 24 mo, %	71* *P* < .0001*	67* *P* < .0001*	44
MMR by 24 mo by Sokal risk group, %			
Low (n = 103, 103, 104)	73	74	53
Intermediate (n = 101, 100, 101)	74	67	44
High (n = 78, 78, 78)	65	56	32
MMR rate among pts with CCyR, %			
At 12 mo (n = 195, 191, 154)	54	50	34
At 24 mo (n = 182, 180, 169)	83	74	53
MR^4 at any time, %	44 *P* < .0001*	36 *P* < .0001*	20
$MR^{4.5}$ at any time, %	26 *P* < .0001*	21 *P* = .0004*	10
$MR^{4.5}$ at any time by Sokal risk group, %			
Low (n = 103, 103, 104)	24	29	10
Intermediate (n = 101, 100, 101)	33	13	15
High (n = 78, 78, 78)	21	21	5
Freedom from progression to AP/BC, (%)			
Estimated rate at 24 mo Excluding CE	99.3 *P* = .0059**	98.1 *P* = .0196**	95.2
Including CE	99.3 *P* = .0003**	97.3 *P* = .0089**	93.2
PFS, %			
Estimated PFS rate at 24 mo	98.0 *P* = .0736**	97.7 *P* = .0437**	95.2
OS, %			
Estimated OS rate at 24 mo	97.4 *P* = .6485**	97.8 *P* = .2125**	96.3
Estimated OS rate at 24 mo considering only CML-related deaths	98.9 *P* = .1930**	98.9 *P* = .0485**	96.7

* Cochran-Mantel-Haenszel test stratified by Sokal vs imatinib.
** Log-rank test stratified by Sokal vs imatinib.

4.3. **[453] Alternating Nilotinib 400 mg twice daily and Imatinib 400 mg once daily as Frontline Treatment of Ph+ Chronic Myeloid Leukemia. A Phase 2 Multicentric Study of the GIMEMA CML Working Party.** *Castagnetti*. Background: Imatinib (IM) 400 mg daily is the standard treatment for Ph+ Chronic Myeloid Leukemia (CML) in early Chronic Phase (ECP). Nilotinib (NIL) is a 2nd generation tyrosine kinase inhibitor (TKI) with superior efficacy to IM (phase 3 ENESTnd trial). NIL has been approved for the frontline treatment of CML in many countries. The treatment with more than one TKI, according to the principles of cancer polychemotherapy, may improve the response rates and may decrease the frequency of drug-resistance. The combination of different TKIs is potentially toxic, difficult to be explored in the ECP setting. The sequential administration of IM and NIL is worth to be investigated because a significant proportion of CML patients treated with a single TKI as monotherapy develops primary or secondary resistance. Aims: To evaluate the response (either cytogenetic and molecular) and the outcome of ECP Ph+ CML patients treated with the sequential administration of NIL and IM. Methods: A phase 2 study was conducted by the GIMEMA CML WP (ClinicalTrials.gov. NCT00769327). NIL was administered first because it has a more rapid therapeutic effect. Schedule: NIL 400 mg twice daily for 3 months; IM 400 mg daily for the next 3 months; then, NIL and IM turning every 3 months, for a total duration of 24 months (study core). The 3-month rotation schedule was respected, irrespectively of temporary discontinuations. The primary end-point was the Complete Cytogenetic Response (CCgR) rate at 12 months. If the conventional cytogenetic analysis resulted not evaluable, a FISH analysis was performed. If one of the 2 drugs was permanently discontinued for adverse event (AE), the patient

remained in study, continuing the treatment with the other drug (except for cardiac AEs). Definitions: Major Molecular Response (MMR): BCR-ABL/ABL ratio < 0,1%^{IS}; failure: according to 2009 ELN recommendations; event: failure, permanent discontinuation of both drug for any reason, patient refusal. All the analysis were performed according to the ITT principle. **Results:** 123 patients have been enrolled in 38 italian hematologic Centers; median age 56 years (range 18-84); 33% low, 45% intermediate and 22% high Sokal score; median follow-up 21 months (at least 12 months observation, 24 months by November 2011). The cumulative CCgR rate by 12 months was 87%; CCgR at each milestone: 68% at 3 months, 73% at 6 months, 67% at 12 months (primary efficacy variable). The cumulative MMR rate by 12 months was 82%, while the rates of MMR at 3, 6 and 12 months were 59%, 62% and 60%, respectively. The discrepancies between cumulative response rates and response at each timepoint were mainly due to the number of patients not evaluable at each timepoint (10% of cytogenetic analysis not evaluable at 12 months) and to protocol discontinuation in stable CCgR and/or MMR. The incidence of hematologic AEs was low. Non-hematologic AEs or lab abnormalities grade > 2 observed in >5 % of patients were as follows: NIL - skin rash, pruritus, bilirubin increase, transaminase increase, lipase increase; IM - fluid retention, periorbital edema. AEs were manageable with appropriate dose adaptations. Four patients (3%) showed a prolongation of the QTcF above 450 msec (none above 500 msec). At the end of the first 12 months, 95 patients (77%) remained on study: 6 and 3 on NIL and IM monotherapy, respectively, 86 on sequential treatment with both drugs; 15% of patients permanently reduced the NIL dose to 400 mg daily and 9% of patients permanently reduced the IM dose to 300 mg daily. During the first year, 28 patients (23%) experienced an event: 10 treatment failure (8%); 2 death in CP (2%); 16 refusal, protocol violation or AE (13%). Six out of the 10 patients who failed the treatment progressed to advanced phase (3 patients: detection of a T315I mutation). **Conclusions**: The cumulative response rates achieved with a sequential administration of NIL and IM seem to be superior to the historical data of IM alone. The response rates at each timepoint, lower than expected, were probably due to the high number of not evaluable patients and to the number of patients not continuing the study despite a stable CCgR/MMR. However, if compared to the excellent results of 2nd generation TKI as monotherapy in ECP CML (single and randomized trials), the present analysis do not support an alternating schedule of NIL and IM as frontline treatment of ECP CML.

4.4. **[454] Efficacy of Frontline Nilotinib Therapy in Patients (Pts) with Newly Diagnosed Philadelphia Chromosome (Ph)-Positive Chronic Myeloid Leukemia in Early Chronic Phase (CML-CP).** *Quintás-Cardama*. Background: In 2005, we initiated a phase II study of nilotinib as 1st line therapy in pts with newly diagnosed CML-CP to investigate the efficacy and safety of nilotinib as frontline therapy for pts with CML-CP. Methods: The primary objective was to estimate the proportion of pts attaining major molecular response (MMR) at 12 months (mo). Pts with untreated CML-CP within 6 mo from diagnosis were eligible and received nilotinib 400 mg twice daily. Results: 100 pts (41% female) have received for a median of 24 mo (range 1 to 72mos). Median age was 49 years (range 17-86). Median WBC, PB blasts, PB basophils, hemoglobin, and platelet count was 42.6, 0%, 2.5%, 12.3, 307, respectively. Five pts (5%) had a variant Philadelphia chromosome and 1 (1%) had deletion of derivative chromosome 9. Seventy-two (72%), 20 (20%), and 8 (8%) pts had low, intermediate, and high Sokal risk score. Among the 102 CP pts who were not in CHR at the start, 100 (98%) achieved CHR (one discontinued after 2 weeks without adverse events). Among 73 CP pts followed for at least 12 mo, 69 (95%) achieved a complete cytogenetic response (CCyR). MMR at 18 mo has been achieved in 51 (89%) pts, including 30 (52%) with a complete molecular response (CMR)(Table 1). The median time to achieve CCyR, MMR was 6 mo each.

	Time on Treatment (months)						
	3	6	12	18	24	36	48
CCyR	78	92	97	98	98	100	100
MMR	40	35	86	89	88	95	95
CMR (BCR-ABL1 ≤0.0032 (IS))	7	74	45	52	65	66	75
Annual rate events (%)			4		0	2	5

Annual rate progression (%)			2		0	0	0
No. Pts evaluable	91	87	66	54	51	37	19

Grade 3-4 thrombocytopenia, neutropenia, and anemia occurred in 9%, 12%, and 6% pts. The most frequent non-hematologic toxicities were rash (62%), pain (57%), and elevated transaminases (45%) and bilirubin (42%). However, grade 3-4 non-hematologic adverse events (possible, probable or suspected relationship only) were rare, including: pain and increased bilirubin (4% each), elevated lipase, fatigue, and elevated transaminases (2% each), and hyperglycemia (1%). One (1%) pt experienced QTc prolongation (grade 2; QTc prolonged from 444msec to 483msec), not associated with arrhythmias and resolved after a brief treatment interruption. Forty-five (45%) pts had transient treatment interruptions (median days off-nilotinib 7 [range 1-68]) and 27 (27%) had dose reductions. Of the patients that were dose reduced, their current or last known dose was either 200mg daily (n=7), 200mg twice daily (n=14), or 400mg daily (n=6). Nineteen (19%) pts terminated nilotinib therapy due to toxicity (n=7), personal reasons or loss to follow-up (n=7), loss of MCyR (n=2), progression to BP (n=2), or death (n=1). Of the pts who discontinued therapy, 3 were tested for BCR-ABL1 mutations; 2 were found to have mutations (F359C and Y253H). The 48 mo probability of EFS (event= loss of CHR, loss of MCyR, AP/BP, or death) is 88%. The annual rate of events during the first 48 mo of follow-up was 4%, 0%, 2%, 5%, and 0% and the rate of transformation 2%, 0%, 0%, and 0%, respectively. The best response achieved on nilotinib by the 2 pts that transformed to BP was CCyR and PCyR, respectively. The overall survival at 48 mos is 96%. One pt died due to stroke, unrelated to nilotinib. No other vascular events have been observed to date. **Conclusion:** Nilotinib 400 mg twice daily induces CCyR in 78% of pts as early as 3 mo and MMR in 86% at 12 mo after the start of therapy, with very low rates of progression to AP/BP and a mild toxicity profile.

4.5. [455] Bosutinib Versus Imatinib in Newly Diagnosed Chronic Phase Chronic Myeloid Leukemia – BELA Trial: 24-Month Follow-up. *Cortes.* Introduction: Bosutinib (SKI-606) is an orally active, dual competitive inhibitor of the Src and Abl tyrosine kinases. The phase 3 BELA study compared bosutinib with imatinib in patients (pts) with newly diagnosed chronic phase (CP) chronic myeloid leukemia (CML). Methods: Pts were randomized 1:1 to open-label oral bosutinib 500 mg/d (n = 250) or imatinib 400 mg/d (n = 252) and stratified by Sokal score risk group (low, medium, high) and geographical region. The primary efficacy endpoint was complete cytogenetic response (CCyR) at 12 mo in the intent-to-treat population. Key secondary and exploratory efficacy endpoints included major molecular response (MMR) at 12 mo, time to CCyR and MMR, duration of CCyR and MMR, time to and incidence of transformation to accelerated/blast phase (AP/BP) CML, event-free survival (EFS), and overall survival. Safety analyses included all treated pts. Results: The median treatment duration was 19.3 mo for bosutinib and 19.5 mo for imatinib; 67% and 74% of pts, respectively, are still receiving therapy. The primary reason for discontinuation of bosutinib was toxicity (23%), while the primary reason for discontinuation of imatinib was disease progression (13%). Rates of CCyR and MMR are shown in the table. The rate of cumulative CCyR by 18 mo was 79% in both arms, and the cumulative rate of MMR by 18 mo was 55% in the bosutinib arm versus 45% in the imatinib arm. Median time to CCyR was faster for bosutinib versus imatinib (12.7 vs 24.6 wk); median time to MMR was also faster for bosutinib versus imatinib (36.9 vs 72.3 wk). Transformation to AP/BP CML while on treatment occurred in 4 (2%) pts on bosutinib and 13 (5%) pts on imatinib. On-study deaths from any cause occurred in 6 (2%) pts receiving bosutinib versus 13 (5%) pts receiving imatinib, and included 5 (2%) and 9 (4%) pts, respectively, who died due to CML progression. Median on-treatment EFS and overall survival were not yet reached for either arm. At 18 mo, the Kaplan-Meier estimates of EFS were 95% for bosutinib versus 91% for imatinib, and the estimates of overall survival were 99% versus 95%, respectively. Bosutinib was associated with higher incidences compared with imatinib of gastrointestinal events (diarrhea [69% vs 22%, respectively], vomiting [32% vs 14%], pyrexia [18% vs 10%], and abdominal pain [13% vs 7%]). In contrast, bosutinib was associated with lower incidences of edema (peripheral edema [4% vs 11%] and periorbital edema [1% vs 14%]) and musculoskeletal events (myalgia [5% vs 11%], muscle cramps [4% vs 22%], and bone pain [4% vs 10%]). Fewer pts on bosutinib experienced grade 3/4 laboratory abnormalities of neutropenia (11% vs 24% with imatinib), while the incidences of grade 3/4 anemia and thrombocytopenia were similar between treatment arms (8% with anemia and 14% with thrombocytopenia). Grade 3/4 liver function test abnormalities occurred more frequently with bosutinib versus imatinib (increased alanine aminotransferase [23% vs 4%] and aspartate aminotransferase [12% vs 3%]). Although common with bosutinib, gastrointestinal events and liver function test abnormalities were typically transient, managed with dose modifications, and not life threatening. Conclusions: The study did not meet the primary endpoint (CCyR at 12 mo); early discontinuation of bosutinib due to adverse events may have contributed to this observed lack of difference. However, bosutinib did result in a higher rate of MMR at 12 mo, faster times to MMR and CCyR, fewer events of transformation to AP/BP CML, and fewer overall and CML-related deaths compared with imatinib, suggesting superiority of bosutinib in pts with newly diagnosed CP CML. In addition, the 18-mo estimates for both EFS and OS currently favor bosutinib. Bosutinib and imatinib were each associated with acceptable but distinct toxicity profiles. Based on these results, bosutinib may offer a new therapeutic option for pts with newly diagnosed CP CML. Minimum of 24 mo of follow-up will be presented for all pts.

Table.

Response	Bosutinib (n = 248)	Imatinib (n = 250)
CCyR		

At 3 mo[a]	50%	25%
At 6 mo[a]	59%	49%
At 9 mo	63%	55%
At 12 mo	70%	68%
At 18 mo	62%	67%
MMR		
At 3 mo[a]	7%	3%
At 6 mo[a]	28%	11%
At 9 mo[a]	35%	19%
At 12 mo[a]	41%	27%
At 18 mo	46%	38%

[a]Statistically significant (*P* value <0.05). *P* values were based on a Cochran-Mantel-Haeszel test stratified by site and geographic region; all time points, with the exception of Month 12, were exploratory.

4.6. **[456] Pegylated Interferon a2a (PegIFN) At the Dose of 45µg Per Week in Combination with Imatinib 400mg Is the Recommended Initial Dose for Patients (pts) with Chronic Phase Chronic Myeloid Leukemia (CML-CP): Results From the French SPIRIT Trial of the French CML Group (FI LMC).** *Johnson-Ansah. Background* :The SPIRIT phase III randomized multicenter open-label prospective trial was designed to compare 4-arm, imatinib 400 mg versus imatinib 600 mg versus imatinib 400 mg + cytarabine at a dose of 20 mg/m²/day in cycles of 28 days, versus imatinib 400 mg + PegIFN at an initial dose of 90 µg/week. The planned molecular analysis after 1 year based on the outcome of 636 pts resulted in a highly significant improvement of superior molecular response (SMR) (0.01 % Bcr-Abl/Abl on IS) of the combination imatinib 400mg-PegIFN (N Engl J Med, 2010). Accrual within the imatinib 600 mg and imatinib 400 mg-cytarabine has been stopped. In the initial cohort of 171 pts who had been treated less than 4 months with the 2 combined agents, major molecular response (MMR: 0.1%) rate was 48%, SMR rate was 23%, and undetectable molecular residual disease (UMRD) was 8%. By contrast, in pts receiving the combined agents longer than 12 months, MMR, SMR and UMRD rates were 82%, 49% and 20% respectively. In order to improve tolerability of the combination, initial dose of PegIFN has been reduced to 45µg/week. The current analysis focuses on the tolerability and efficacy of the reduced dose of PegIFN as compared to the initial planned dose of 90µg/week. *Patients and methods*: As of December 31st 2010, date for closing accrual, 789 pts have been included, 445 pts within the imatinib 400mg and imatinib 400mg+PegIFN arms. The high proportion of PegIFN discontinuation during the first year prompted an amendment that recommended reducing the initial dose down to 45ug/week at enrolment. For these pts the weekly dose of PegIFN was increased up to 90µg after 2 months of treatment with the combination of PegIFN 45µg plus Imatinib 400mg, if the hematological and non-hematological tolerance was acceptable. Out of the 221 pts assigned to imatinib plus PegIFN, 171 received 90µg and 50 received 45µg/week of PegIFN, both groups being similar with a median age of 51 and 48 years respectively. Molecular biology was centralized, samples being collected every 4 months, with a karyotype recommended yearly. Data of parameters of effectiveness and tolerance were collected even after stopping treatment protocol. Adverse events (AE) considered for the analysis are those which led for PegIFN dose adjustment. *Results*. At the time of the analysis, 30% of pts were still on PegIFN. Table 1 describes the AE which occurred during the two consecutive periods of the trial (before and after the amendment).

	AE before the amendment (n=171)			AE after the amendment (n=50)		
	All grade (%)	G1-2 (%)	G3-4 (%)	All grade (%)	G1-2 (%)	G3-4 (%)
Hematologic toxicity	68	14	54	45	18	27

Flu-like syndrome	7	5	2	4	2	2
Skin toxicity	7	2	5	2	2	0
Neuropsychiatric syndrome	9	5	4	6	2	4
Liver toxicity	1	0.5	0.5	6	2	4
other	3	1	2	6	2	4

Hematologic toxicity was predominant within the PegIFN arm (68% pts suffering all grade including 54% with G3-4) leading to 40% of permanent discontinuation of PegIFN. However, only a small proportion of neuropsychiatric syndrome (including depression, chronic fatigue and insomnia) and flu-like syndrome were observed and no cardiac event occurred with PegIFN at the dose of 45µg/week. Rate of all grade hematologic toxicity decreased from 68% to 45% of pts after the amendment. Of interest the reduced dose of PegIFN had a significant impact on the adherence to the treatment. During the first 6 months before and after the amendment, 40% and 10% of pts discontinued PegIFN respectively. Thus after the amendment 90% received at least 6 months of PegIFN. Of note the reduced dose of 45µg/week resulted in a similar response rate as compared to the initial planned dose of 90µg. The MMR rates at 6 months were before and after the amendment 36.3% (95%CI: 29.1-49.3) and 36% (95%CI: 22.0-50.8) respectively. The corresponding number for SMR rates at 6 months were 9.9% (95%CI: 5.9-15.4) and 10% (95%CI: 3.3-21.8) respectively. Finally for 222 pts, imatinib 400mg alone resulted in less responses at 6 months with MMR and SMR rate of 19.3% (95CI: 14.3-25.0) and 5.8% (95CI: 3.1-0.7) respectively. The combination of imatinib and PegIFN has been shown in this trial as an effective combination for increasing the rate of molecular responses compared to imatinib alone. The lower dose of PegIFN (45 mg/week) resulted in less early toxicity. It will allow the combination to be given together for a longer period of time and preserve the increased antitumor efficacy. An update with a minimum of 12 months of follow-up of the 50 pts will be presented.

5 Therapy: New Drugs, New Procedures [601-606]

5.1. **[601] A Phase 1 Study of DCC-2036, a Novel Oral Inhibitor of BCR-ABL Kinase, in Patients with Philadelphia Chromosome Positive (Ph^+) Leukemias Including Patients with T315I Mutation.** *Cortes*. *Background.* DCC-2036 is a novel and potent tyrosine kinase inhibitor (TKI) which binds to a novel region called the switch pocket, thereby preventing BCR-ABL from adopting a conformationally active state. Efficacy against multiple imatinib-resistant BCR-ABL mutants has been demonstrated both *in vitro* and *in vivo* (Chan et al., Cancer Cell 2011;19:556). Importantly, DCC-2036 retains full potency against the T315I mutant in preclinical efficacy studies. *Methods.* This study was designed to find the maximal tolerated dose (MTD) of DCC-2036 when administered daily as a single-agent on a 28-day cycle. Eligible patients included adults with Ph+ CML/ALL who were refractory/intolerant to ≥2 TKI's or were T315I positive. Initially DCC-2036 capsules were administered orally once daily (QD) at increasing dose levels. Only 1 patient was enrolled in each of the lowest dose cohorts of 57mg QD and 114 mg QD. For higher doses, 3- 6 patients were enrolled into each ascending dose cohort with standard dose limiting toxicity (DLT) rules evaluating safety in cycle 1 to determine dose escalation. A transition from unformulated capsules (C) to formulated tablets (T) occurred after the 1200 mg QD dose level. Paired blood samples were obtained for PK and PD assessments. *Results.* 30 patients (16 males, 14 females; median age 59, range 31 – 80) with CML including 19 in Chronic (CP); 8 in Accelerated (AP) and 3 in Blast (BP) Phase were enrolled. Enrolled patients had received 1-6 prior CML treatments, and 11 patients had the T315I mutation. To date, a total of 212.5 (median 5.6; range 0.2 – 23.4) 28-day cycles were administered over 10 dose levels either as C (7 dose levels) or T (3 dose levels). The 7 C dose levels were studied first and included 57 mg QD through 1200 mg QD. Following transition to T, evaluation continued with 100 mg QD, 100 mg twice daily (BID), and 200 mg BID. Two reversible DLTs (Grade 3 peripheral neuropathy and Grade 4 lower extremity weakness) occurred during the initial treatment cycle at the 200 mg T BID dose level. Evaluation of 6 patients at the 150 mg T BID dose level determined that dose to be the MTD. Preliminary safety data show that other Grade (Gr) 3/4 adverse events (AEs) were Gr 3 slurred speech and Gr 3 eruptive nevi. Gr 1/2 AEs included dry mouth, constipation, diarrhea, paresthesias, and retinal vein occlusion. There was 1 case of Gr 2 pancreatitis that recurred on rechallenge in a

patient with previous pancreatitis with nilotinib. Preliminary responses include one major molecular response in a CP patient with T315I mutation who started on capsules and transitioned to 100 mg T QD. There was one complete cytogenetic response in a CP patient at 100 mg T BID, and one partial cytogenetic response in a CP patient who started on capsules and transitioned to 100 mg T BID. One patient with AP CML and T315I mutation had a complete hematologic response at 450 mg C QD. Another patient with AP CML had a partial hematologic response after receiving 200 mg BID for 1 cycle and then downdosing to 100 mg T BID. Four out of 8 patients receiving 100 mg tablets and evaluable for efficacy (completed 3 cycles of treatment) had responses. PK results indicate dose-related, nonlinear increases in both peak plasma concentration (Cmax) and exposure (AUC). PD results reveal both acute and steady state post-treatment reductions in phospho-protein levels on Days 1 and 8. Marked reductions in pSTAT5 and pCRKL have been observed in subjects with both CP and AP and appear to be required for clinical response. *Conclusion:* The MTD of DCC-2036 tablets is 150 mg BID. Preliminary results suggest that DCC-2036 is well tolerated and has anti-leukemia activity in subjects with refractory CML and T315I positive disease. PD results are consistent with inhibition of BCR-ABL signaling in this first-in-man study of a switch pocket tyrosine kinase inhibitor.

4 of 19 in CP
One patient had all 3 TKs CCR with V299L. However got RVO and had to stop treatment.

5.2. **[602] Subset Analysis of Response to Treatment of Chronic Phase CML in a Phase 1 Study of Ponatinib in Refractory Hematologic Malignancies.** *Cortes. Background:* Ponatinib is a potent, oral, pan-BCR-ABL inhibitor active against the native enzyme and all tested resistant mutants, including the uniformly resistant T315I mutation. Initial findings of a phase 1 trial in patients (pts) with refractory hematologic malignancies have been reported. The effect of duration of treatment, prior treatment, and mutation status on response to treatment was examined in CML chronic phase (CP) pts who responded to ponatinib. *Methods:* An open-label, dose escalation, phase 1 trial of ponatinib in pts with hematologic malignancies is ongoing. The primary aim is to assess the safety; anti-leukemic activity is also being investigated. Pts resistant to prior treatments or who had no standard treatment available were enrolled to receive a single daily oral dose of ponatinib (2 mg to 60 mg). Subset analyses of factors impacting cytogenetic and molecular response endpoints (MCyR and MMR) were performed for pts with CP-CML. Data are presented through April 15, 2011. *Results:* In total, 81 pts (54% male) received ponatinib. Overall, 43 pts had CP with 34 ongoing at analysis. MCyR was observed as best response in 31/43 (72%), 27 (63%) CCyR. The median time to MCyR was 12 (3 to 104) wks. Response rates were assessed by duration of treatment (1 pt in CCyR at entry was excluded; 6 pts in PCyR had to achieve CCyR). At the 3 month assessment, 22/42 (52%) CP pts achieved MCyR; at 6 months, 24/42 (57%); at 12 months, 29/42 (69%) had MCyR. The impact of prior treatment on response and time to response was assessed. 42 pts (98%) had >2 prior TKIs and 28 (65%) ≥3 prior TKIs, including investigational agents. Of approved TKIs, all pts were previously treated with imatinib, 19 dasatinib or nilotinib after imatinib, and 21 both dasatinib and nilotinib after imatinib. MCyR rate decreased with number of prior TKIs (2 prior TKIs 13/14 [93%], ≥3 prior TKIs 17/28 [61%]) and number of approved TKIs (imatinib followed by dasatinib or nilotinib 17/19 [90%], or by both dasatinib and nilotinib 12/21 [57%]). Time to response was prolonged in pts more heavily treated with prior TKIs. Median time to MCyR increased with the number of prior TKIs and approved TKIs (2 TKIs 12 wks, ≥3 TKIs 32 wks). The effect of mutation status on response and time to response was also evaluated. At entry, 12 pts had the T315I mutation, 15 had other BCR-ABL kinase domain mutations, 12 had no mutations detected, 4 did not allow sequencing. MCyR response rate for CP pts with T315I was 11/12 (92%); for other mutations, 10/15 (67%); and no mutation, 7/12 (58%). Similarly, mutation status had an impact on time to response: median time to MCyR was 12 wks for those with T315I or other mutations and 32 wks in resistant pts with no mutation. All CP patients were evaluable for MMR. At analysis, MMR was 17/43 (40%). MMR rate was inversely related to number of prior TKIs (2 TKIs 10/14 [71%], ≥3 TKIs 6/28 [21%]), approved TKIs (imatinib followed by dasatinib or nilotinib 12/19 [63%], or by both dasatinib and nilotinib 4/21 [19%]), and was higher for T315I pts (7/12, 58%) and those with other mutations (7/15, 47%) compared with no mutation (2/12, 17%). Median time to MMR for CP pts was 97 wks; median time to MMR was shorter for pts who were less heavily treated (2 prior TKIs 24 wks) and those with T315I or other mutations (63 wks). *Conclusion:* In this subset analysis of the phase 1 data, ponatinib had substantial activity in all subgroups analyzed. Time on treatment, less prior therapy and kinase domain mutations were associated with higher response rates and early responses in CP pts. Cytogenetic responses improved over the first 12 months of treatment and were higher in less heavily treated pts.

CCR 27 of **43 patients (**63)
MMR 19 (44)
9/12 (75%) had CCR with T315I
Responses appear to improve up to 12 months.
Of 31 patients with MCR, 29 of them remained on therapy and sustained their response.
Overall 72% MCR, 44% MMR
Better if fewer prior therapies shorter time since diagnosis and KD mutations

5.3. **[603] Discontinuation of Imatinib in Patients with Chronic Myeloid Leukemia Who Have Maintained Complete Molecular Response: Update Results of the STIM Study.** *Mahon.* Background Imatinib treatment significantly improves survival in patients (pts) with CML. We previously demonstrated that Imatinib could be safely discontinued in pts with a complete molecular response (CMR) of at least 2 years duration and reported an interim analysis on 69 pts having at least 12 months of follow-up (FU) (Lancet oncology, 2010;11: 1029-1035). Little is known about whether treatment can safely be discontinued in the long term. The FU of this pt population is therefore crucial. Herein we aim to present the updated results from the first 100 pts included in the STIM study with a longer FU. Methods In this multicentre, non-randomized Stop

Imatinib (STIM) study, imatinib (of >2 years duration) was discontinued in CML pts with who were aged 18 years and older and in CMR sustained for at least 2 years. Pts that had undergone immunomodulatory treatment (apart from interferon α), treatment for other malignancies, or allogeneic haematopoietic stem-cell transplantation were excluded. Rate of relapse was assessed by use of RT-PCR and was defined as positivity of BCR–ABL transcripts in quantitative RT-PCR with a ratio of BCR–ABL to ABL of 0.001 or more, as confirmed by a second analysis point, indicating the increase (at least 1log) in relation to the first analysis point at two successive assessments. Quantitative RT-PCR for *BCR–ABL* transcripts from peripheral blood was performed every month during the first year and every 2 months thereafter. Beyond 2 years, molecular biology FU was performed every 3 months. Results From July 9, 2007, to Dec 17, 2009, 100 pts (48 men, 52 women) with CML and a median age of 63 years (range 29–80) were recruited in the STIM trial. The median FU of the first 100 pts enrolled was 30 months (range 9-45) with a mean of 30 months. After imatinib was discontinued, a molecular relapse occurred in 61 pts with 58 relapses occurring during the first 7 months and 3 late relapses at month 19, 20 and 22, respectively. The overall probability of maintenance of CMR at 24 and 36 months was 39% (95% CI 29-48). We confirmed that all patients were sensitive to an imatinib re-challenge. Among the 61 with molecular recurrence, 56 regained CMR after imatinib re treatment. A median time of 4 months (range 0–21) was necessary for CMR to recur. Five pts did not return to CMR, 4 pts were continuously free of treatment with a median BCR-ABL level of 0.15% (0.05 to 0.3) at last evaluation and one received dasatinib due to a BCR-ABL level of 6.6%, i.e corresponding to a loss of a complete cytogenetic response. Among the 39 pts without confirmed molecular relapse, 5 exhibited clearly a fluctuation in BCR-ABL transcripts levels with a median FU of 22 months (6-35). Sokal risk group was available for 95% of pts and among the 11 pts with high sokal score 10 relapsed. The probability to be in stable CMR after discontinuation was significantly better for the low risk group (55% at 24 months, p<0.001) as compared to intermediate and high risk group. Using multivariate analysis, we confirmed that Sokal risk score (low *vs* intermediate *vs* high; p=0.0009) and Imatinib therapy duration (<60 months *vs* ≥60 months p=0.0183) were 2 independent prognostic factors for prediction of molecular relapse after imatinib cessation. Taking into account the cost of imatinib and the number of months without treatment in the total study population at last analysis, the savings within the STIM trial were estimated at 4 million Euros. Conclusion Imatinib can be safely discontinued in pts with a CMR of at least 2 years duration. This category of pts might be cured from CML with tyrosine kinase inhibitors. Cure may not require the eradication of residual leukemic stem cells since it has been reported that the more sensitive PCR on DNA or RNA to assess CMR does not allow the prediction of relapse after discontinuation. In addition with this longer FU, positive fluctuation PCR results do not mean CML relapse or progression. It is thus conceivable that below a threshold of residual leukemic cells the inherent nature of the disease (illustrate by the Sokal score) and the duration of therapy are probably the most important factors to predict relapse after discontinuation.

5.4. **[604] Discontinuation of Dasatinib or Nilotinib in Chronic Myeloid Leukemia (CML) Patients (pts) with Stable Undetectable Bcr-Abl Transcripts: Results From the French CML Group (FILMC).** *Rea*. Background: Imatinib, the first tyrosine kinase inhibitor (TKI) directed against the Bcr-Abl oncoprotein, has dramatically improved outcomes for pts with CML. Dasatinib and nilotinib, 2 highly potent second generation (2G)-TKI, have been historically licensed for the treatment of pts with resistance or intolerance to imatinib. They have recently received approval in the frontline setting in chronic phase (CP)-CML. Despite the outstanding efficacy of these drugs, their curative potential remains uncertain. Most TKI-treated pts retain residual leukemic cells detected by means of RTQ-PCR and termination of TKI therapy under such circumstances usually leads to disease relapse. Consequently, it is believed that most CML pts require a lifelong TKI treatment. On the contrary, stopping TKI may be envisaged in pts with stable undetectable molecular residual disease (UMRD), as suggested by recent results from the STop IMatinib trial (Mahon *et al.* Lancet Oncol. 2010), and our observation that dasatinib could be safely stopped in a pt with a stable UMRD suffering from drug-induced pleural effusion (Cony-Makhoul, *et al.* unpublished). Aims: We asked whether 2G-TKI could be ceased in CML pts with a stable UMRD. The primary objective of our work was to evaluate the risk of loosing major molecular responses (MMR: *BCR-ABL/ABL* internationally standardized (IS) ratio ≤ 0.1%) by 6 months. Methods: Pts aged at least 18 years with CP-CML and UMRD were proposed dasatinib or nilotinib discontinuation provided that (1) no prior progression to accelerated phase or blast crisis occurred (2) UMRD was sustained on continuing therapy. UMRD was defined by undetectable Bcr-Abl using internationally standardized RTQ-PCR testing performed in local laboratories, providing that at least 20 000 copies of the control gene had been amplified. After 2G-TKI discontinuation, Bcr-Abl transcripts were quantified monthly during the first 6 months and every 2 to 3 months thereafter. Dasatinib or nilotinib were advised to be re-introduced upon loss of MMR. Results: As of August 1, 2011, 25 pts agreed to stop therapy. The results presented here focus on the subgroup of 16 pts with a minimum follow-up (FU) of 6 months (median 15, range: 7-21). These were 9 females and 7 males, with a median age of 59 years (34-81). The Sokal risk group was low in 11/16 (68.75%), intermediate in 2/16 (12.5%), high in 1/16 (6.25%) and unknown in 2/16 (12.5%). Dasatinib (n=9) or nilotinib (n=7) had been administered owing to imatinib grade 2 hematologic or grade 2 to 4 non hematologic intolerance (n=13), secondary imatinib resistance (n=1) or as the frontline drug (n=1). At start of 2G-TKI, 1 pt was in CP, 1 had a complete hematologic response only, 2 had a partial cytogenetic response, 3 had a complete cytogenetic response but lacked MMR, 4 had a MMR with detectable Bcr-Abl transcripts and 5 had a UMRD. The median time on 2G-TKI therapy prior to discontinuation was 32 months (21-56). The median duration of sustained UMRD was 27 months (21-64). Subsequently, MMR was lost in 31.25% (5/16) pts after a median time off-therapy of 4 months (1-5). Treatment was restarted in 4 of these and in an additional pt without MMR loss but showing a detectable MRD on 2 consecutive assessments. Both MMR and UMRD were rapidly regained upon 2G-TKI re-introduction. Eleven pts remained off-therapy at the last follow-up after a median of 13 months (7-20), among which 10 with either a stable UMRD or weakly detectable Bcr-Abl transcripts on one or more occasions. Gender, age, Sokal risk group, type of 2G-TKI, total duration of continuous TKI treatment, duration of 2G-TKI therapy and of UMRD prior to treatment discontinuation did not markedly differ between pts who lost MMR and those with treatment-free persistent MMR but these results may be taken with caution due to the small size of our cohort. Conclusion: 2G-TKI may be safely discontinued in CML pts with a long-lasting UMRD under strict molecular monitoring conditions. Importantly, the emergence of a low level of detectable residual disease below the MMR threshold after 2G-TKI withdrawal may not automatically herald CML relapse and may not preclude the possibility to remain

treatment-free. A longer follow-up is required to ascertain whether CML will recur. Our study provides a reasonable basis for subsequent large scale prospective trials. Updated results based on a minimal 6 month-FU of the whole cohort will be presented.

5.5. **[605] The Natural History of RTQ-PCR Levels After the Achievement of Complete Molecular Remission (CMR): Implications for 'Stopping' Studies.** *Milojkovic.* Variations in RTQ-PCR estimations of BCR-ABL1 transcript numbers between laboratories have resulted in recognised difficulties in interpreting results and have led to a global effort of harmonisation via an international reporting scale (IS). Currently this is achieved in a limited number of laboratories worldwide by exchange of samples and will hopefully be replaced by the production of internationally accredited reference reagents. Differences in the limits of sensitivity of assays in different laboratories pose particular problems in the definition and interpretation of molecular negativity, so-called complete molecular remission (CMR), leading some investigators to suggest distinctions between assays capable of detecting 4, 4.5 and 5 log reductions in tumour load and introducing the terms CMR4, CMR4.5 and CMR5. These definitions take on particular relevance when designing studies of de-escalation and/or stopping tyrosine kinase inhibitor (TKI) therapy. In the French STIM trial, criteria for stopping were relatively stringent in that patients were required to have at least 5 results of RTQ-PCR negativity in their local laboratory sustained over at least 2 years and confirmed on one further occasion in the centralised laboratory. Negative results of BCR–ABL1 amplification were reported only if the RNA was of good quality and quantity (50 000 copies of normal ABL1). Subsequently several groups are designing similar studies. As our ability to stop treatment must in large part be determined by the level of residual disease at the time of cessation, it is important to have robust definitions of CMR. We maintain a comprehensive database of all our CML patients on TKI. For chronic phase this database now contains 521 patients (273 [52%] male) of median age 48 yrs (range 13-86). 212 patients received interferon prior to TKI therapy. The median follow up for surviving patients is 76 mths (range15-137). 88 (37 [42%] male) of these patients have achieved RTQ-PCR molecular negativity on more than one occasion and prompted us to identify the proportion that would satisfy entry criteria for a stopping study and hence the natural history of RTQ-PCR results in such patients. Confirmed complete molecular response (cCMR) was defined as two consecutive samples with no detectable transcripts at least 4 weeks apart with an ABL1 control >40,000 copies (median ABL1 control in the CMR samples was 84,000 copies). 64 patients met our criteria for cCMR, the remaining 24 patients had at least two negative results but never consecutively. 56 patients achieved cCMR on their first line TKI (imatinib in all but 2). Times from diagnosis to MMR and cCMR in this cohort were a median of 24 (range 3-77) and 46 mths (range 5-118) respectively. The median time from MMR to cCMR was 26 mths (range 0-89). Excluding 8 patients in whom follow-up since cCMR is less than 24 months the median duration from cCMR is 53 mths (range 24-113), Only one patient has subsequently lost MMR confirming the excellent prognosis of this cohort. However, only 10 patients (21%) have sustained RTQ-PCR negativity over a 2 year period that would deem them eligible for a STIM-equivalent study. If we were to define a less stringent CMR4.5 as a BCR-ABL ratio of 0.0032 in the international scale the number of eligible patients increases to 18/48 (37.5%). If we applied CMR4.5 to the 24 patients without consecutive RTQ-PCR results a further 3 patients would meet the criteria for a stopping study, total 21/88 (24%). In conclusion the numbers of patients eligible for stopping studies confined to sustained cCMR is relatively few although we cannot exclude the possibility that some patients were not entirely compliant. Although not proven, reducing the stringency of the definition of CMR is likely to lead to higher relapse rates in subsequent stopping studies than in the original STIM trial. This must be considered when interpreting the results of first-line second generation TKI where the rates of achievement of MMR and CMR may be higher than with imatinib. In these studies CMR may not be synonymous with a 50% chance of discontinuing treatment permanently and future studies might more appropriately consider strategies of de-escalation rather than cessation.

5.6. **[606] Complete Molecular Response (CMR) Rate with Nilotinib in Patients (pts) with Chronic Myeloid Leukemia in Chronic Phase (CML-CP) without CMR After ≥ 2 Years on Imatinib: Preliminary Results From the Randomized ENESTcmr Trial of Nilotinib 400 Mg Twice Daily (BID) Vs Imatinib.** *Hughes. Background:* Recent studies have demonstrated that about 40% of very highly selected CML-CP pts treated with imatinib achieve durable CMR and may be able to cease therapy without disease recurrence. However, most CML pts don't achieve CMR on imatinib even with long-term therapy. Results from ENESTnd demonstrated that significantly more patients achieved MMR (≤ 0.1%^{IS}), CMR^{4} (≤ 0.01%^{IS}), and CMR$^{4.5}$ (≤ 0.0032%^{IS}) with nilotinib vs imatinib by 12, 18, and 24 months (mo). No pt in ENESTnd who achieved CMR$^{4.5}$ has progressed to AP/BC. In this study we asked whether pts on long-term imatinib would be more likely to achieve undetectable BCR-ABL levels if they switched to nilotinib, allowing for participation in potential cessation studies in the future. *Methods:* This open label, 1:1 randomized, prospective, multi-center, phase 3 study enrolled 207 pts with CML-CP who had achieved a complete cytogenetic response (CCyR) but were still BCR-ABL positive by RQ-PCR after ≥ 24 mo on imatinib. CMR (primary endpoint) was defined as undetectable BCR-ABL by RQ-PCR where there was no detectable BCR-ABL with a sample sensitivity of ≥ 4.5-logs. CMR^{4} and CMR$^{4.5}$ were defined as BCR-ABL levels of ≤ 0.01% and ≤ 0.0032% expressed on the International Scale (IS), respectively and included patients with undetectable BCR-ABL levels with high sample sensitivity (4 or 4.5 logs). Patients were randomized to nilotinib 400 mg BID vs continuing imatinib 400 or 600 mg daily (same dose as at study entry.) The randomization was stratified by prior use of IFN (none, ≤ 12 mo, > 12 mo) and prior duration of imatinib therapy (> 36 mo or ≤ 36 mo). The primary endpoint was the rate of confirmed best cumulative CMR by 12 mo of study therapy with nilotinib or imatinib. Secondary objectives included the kinetics of CMR at different timepoints, duration of CMR, progression-free survival, and overall survival in both arms. During the study, RQ-PCR for BCR-ABL was performed at baseline (BL) and every 3 mo and expressed on the IS in national reference laboratories in Australia, Brazil, and Canada. For this report: BL, 6 mo, and 12 mo results were analyzed in a central laboratory in Australia. Assessment of the primary efficacy endpoint has not been completed for 2% of the randomized pts; unblinded data will be available for all pts and will be presented by treatment arm. *Results:* BL results were available for 205/207 randomized pts. Overall, 56% of pts had no prior IFN exposure, while 21% and 23% had IFN exposure of ≤ 12 mo or > 12 mo, respectively; 81% of pts had been on imatinib > 36 mos. At BL, 153 pts (74%) were known to be in MMR and 54 pts (26%) had < MMR (including 2 pts with missing PCR samples); 53 pts (26%), and 20 pts (10%) had CMR^{4} or CMR$^{4.5}$ (with detectable BCR-

ABL) at BL, respectively. Overall, 67 pts (32%) had at least a half-log reduction in BCR-ABL levels from BL by 12 mo. Of the 52 pts (25%) known not to have MMR at BL, 24 pts (46%) achieved MMR or better by 12 mo in the study. To date, no pt experienced a loss of MMR or CCyR on study. By 12 mo, 50% of pts with a molecular assessment had CMR^4 and 27% had $CMR^{4.5}$. Of the 152 pts who did not have CMR^4 at baseline, 30% had achieved CMR^4 (unconfirmed) by 12 mo. Of the 185 pts who did not have $CMR^{4.5}$ at baseline, 21% had achieved $CMR^{4.5}$ (unconfirmed) by 12 mo. Undetectable levels of BCR-ABL transcripts (with a test sensitivity of ≥ 4.5 log), were achieved by 12 mo by 12% of pts who did not have undetectable BCR-ABL transcript levels at BL. By 12 mo, 20 (10%) pts discontinued study (none due to progression, 1 due to death). Grade 3/4 adverse events were uncommon. *Conclusions:* ENESTcmr is the first phase 3 randomized study in CML-CP pts to assess the achievement of CMR as the primary endpoint. Unblinded results from this ongoing study will be presented and will provide information on the ability of pts to achieve confirmed CMR on nilotinib vs imatinib following extended treatment with imatinib. These aggregate data demonstrate that 12% of pts achieved undetectable BCR-ABL levels by 12 months after study entry. This is in contrast to observations in previous trials where the increase in the proportion of pts with undetectable BCR-ABL levels over time is more gradual. Further follow-up will identify pts with sustained CMR over time, which may offer these pts an opportunity to discontinue therapy.

6 Therapy: Prediction of Response [781-786]

6.1. **[781] Second Line Therapy with Second Generation TKI After Intolerance to Imatinib Based Treatments Showed High Overall Survival in Contrast to Second Line Therapy After Resistance; Results of the Randomized CML Study IV.** *Saussele. Introduction*: Data on second line therapy with second generation tyrosine kinase inhibitors (TKI) in CML treatment were generated mainly from phase II/III industry initiated trials (Review Hehlmann Exp Op. 2011). 24-month overall survival (OS) varies between 88% and 94% after intolerance and/or resistance to imatinib for chronic phase (CP) and between 67% and 72% for accelerated phase (AP) or blast crisis (BC). Intention to treat analyses including outcome of patients after discontinuation of first line therapies have not been available as yet. We thought to evaluate overall and progression-free survival (OS and PFS) of imatinib intolerant vs. resistant patients under second line TKI with long-term follow-up within an investigator initiated trial. *Methods:* We analyzed data of the German CML study IV, a randomized 5-arm trial to optimize imatinib therapy on an intention to treat basis. According to protocol, follow-up of patients on and after second generation TKI after imatinib intolerance and/or resistance was continued for OS and PFS. Analysis of PFS was only relevant, if intolerance and resistance to imatinib therapy occurred while a patient was still in chronic phase (CP). Patients were censored at the time of allogeneic stem cell transplantation (allo-SCT). *Results:* From July 2002 to December 2010, 1,502 patients with Philadelphia chromosome and /or BCR-ABL positive CML in CP were randomized. 129 patients of the "imatinib after interferon arm" and 36 other patients had to be excluded (14 due to incorrect randomization or withdrawal of consent, 22 with missing baseline information). 1337 were randomized to primary imatinib treatment (imatinib 400 mg vs. imatinib 800 mg vs. imatinib in combination with either interferon alpha or araC). Of these, 234 (17%) discontinued imatinib therapy. 156 patients were treated with 2nd generation TKI, 61 were directly referred to allo-SCT, 17 patients received other regimens (including interferon alpha only or hydroxyurea). 120 of 156 patients started second generation TKI therapy (nilotinib, n=41, dasatinib, n=75, bosutinib, n=2, nilotinib and dasatinib, n=2) within 3 months after stopping imatinib, received treatment for at least one week and were evaluable for PFS and OS. 36 patients received second TKI later (median 10 months, range 3.5-61.4). Median age was 50 years (range 16-78), 42.5% were female. 48 patients were intolerant, 48 failed imatinib within CP and 24 after loss of CP (accelerated phase, n=10, blast crisis, n=14). Median time to second generation TKI was 17 months (range 1.4-97 months) and median follow-up after start of second-line TKI 31 months (range 0.2-71 months). Risk stratification according to the EUTOS Score was high in 20 patients (17%) and low in 94 patients (78%) and unknown in 6 patients (5%). OS for all 120 patients 3 years after start of second generation TKI was 73%, 96% for intolerant and 80% for resistant patients in CP and 19% for resistant patients in advanced disease (s. Fig. 1). According to EUTOS score, 3-year OS was 78% for low and 56% for high risk patients. Probability of PFS of the 96 patients in 1^{st} CP after 3 years was 96% for intolerant and 76% for resistant patients. After 2nd generation TKI, 18 patients received an allo-SCT: all were in CP, 2 patients after imatinib intolerance, 16 patients after imatinib resistance. *Conclusion*: Survival on second generation TKI is high for imatinib intolerant patients in first CP but much lower for resistant patients in first CP or for patients with advanced disease phases. Alternative treatment strategies are warranted for these patient groups.

6.2. **[782] Impact of Additional Cytogenetic Alterations At Diagnosis on Prognosis of CML: Long-Term Observation From 1151 Patients of the Randomized CML Study IV.** *Fabarius.* Introduction: Current evidence indicates that acquired genetic instability in chronic myeloid leukemia (CML) as a consequence of the t(9;22)(q34;q11) and the resulting BCR-ABL fusion causes the continuous acquisition of additional chromosomal aberrations (ACA) and mutations and thereby progression to accelerated phase and blast crisis (BC). Around 10 -12% of patients in chronic phase (CP) CML have ACA already at diagnosis. During the course of the disease this number rises to 80% in BC. Acquisition of ACA during treatment is considered as a poor prognostic indicator, whereas the impact of ACA at diagnosis is controversial. Patients and methods: Clinical and cytogenetic data of 1151 out of 1311 patients with Philadelphia and BCR-ABL positive CP CML randomized until 2009 to the German CML-Study IV were investigated in a prospective study. There were 459 females (40%) and 692 males (60%). Median age was 53 years (range, 16-88). All patients were treated with imatinib alone or in combination with interferon alpha or araC. The impact of ACA at diagnosis on time to complete cytogenetic and major molecular remission (CCR, MMR) and progression-free and overall survival (PFS, OS) was investigated. Written informed consent was obtained from all patients prior to entering the study. Results: At diagnosis 1003/1151 patients (87%) had the standard t(9;22)(q34;q11) only and 69 patients (6.0%) had a variant t(v;22). In 60 of 69 patients with t(v;22), only one further chromosome was involved in the translocation, in 7 patients two, and in 2 patients three further chromosomes were involved. Seventy-nine patients (6.9%) had ACA. Of these, 38 patients (3.3%) lacked the Y chromosome (-Y) and 41 patients (3.6%) had ACA except -Y. Sixteen of the 41 patients had major-route ACA (+8, i(17)(q10), +der(22)t(9;22)(q34;q11), ider(22)(q10)t(9;22)(q34;q11)) and 25 minor-route ACA [e.g. t(3;12), t(4;6), t(2;16), t(1;21)]. In

patients with major-route ACA, trisomy 8 was the most frequent additional alteration (n=9). +der(22)t(9;22)(q34;q11) was observed in six patients, isochromosome (17)(q10) in five patients and ider(22)(q10)t(9;22)(q34;11) in three patients. After a median observation time of 5.3 years for patients with t(9;22), t(v;22), -Y, minor- and major-route ACA median times to CCR were 1.01, 0.95, 0.98, 1.49 and 1.51 years, to MMR 1.40, 1.58, 1.65, 2.49 and > 7 years, 5-year PFS 90%, 81%, 88%, 96% and 50% and 5-year OS 92%, 87%, 91%, 96% and 53%, respectively. In patients with major-route ACA times to CCR and MMR were longer. PFS and OS were shorter (p<0.001) than with standard t(9;22)(q34;q11). Loss of Y chromosome had no influence on time to CCR or MMR, PFS and OS. Conclusion: We conclude that the prognostic impact of additional cytogenetic findings at diagnosis of CML is heterogeneous and consideration of their types may be important. Major-route ACA identify a small group of patients with significantly poorer prognosis as compared to all other patients requiring early and more intensive intervention such as stem cell transplantation.

6.3. **[783] Molecular and Cytogenetic Response After 3 Months of Imatinib Treatment Is Predictive for the Risk of Disease Progression and Death in Newly Diagnosed Chronic Myeloid Leukemia Patients – a Follow-up Analysis of the German CML Study IV.** *Hanfstein. Introduction:* The advent of second generation tyrosine kinase inhibitors (TKI) in the front line treatment setting of chronic myeloid leukemia (CML) has tightened the evaluation of imatinib response. Early assessment of response markers might identify slow responders harboring a BCR-ABL positive clone with an inferior susceptibility to tyrosine kinase inhibition. This group of patients could benefit from an early dose escalation or a change of treatment to a second generation TKI thus avoiding the risk of disease progression. Therefore we sought to evaluate the impact of molecular and cytogenetic response levels after 3 months of imatinib treatment on the further course of disease. *Patients and methods:* A total of 1,340 patients (median age 52 years, range 16-88, 40% female) were included into the randomized German CML study IV and treated with an imatinib based therapy consisting of imatinib 400 mg/d (n=381), imatinib 800 mg/d (n=399) and combinations of standard dose imatinib with interferon alpha (n=402) and low-dose cytarabine (n=158). Median follow-up was 4.7 years (range 0-9). Molecular response after 3 months was assessed in 743 patients, cytogenetic response in 498 patients. The BCR-ABL expression was determined by quantitative RT-PCR and standardized according to the international scale (BCR-ABL IS). Only patients expressing typical BCR-ABL transcripts (b2a2, b3a2, b2a2 and b3a2) were considered. Cytogenetic response was determined by conventional metaphase analysis. Disease progression was defined by the incidence of accelerated phase, blastic phase or death from any reason. A landmark analysis was performed for progression free survival (PFS) and overall survival (OS). *Results:* Disease progression was observed in 149 patients (11.1%), 127 patients died (9.5%). After 3 months of treatment the median BCR-ABL IS was 2.6% (0-100), the median proportion of Philadelphia chromosome positive metaphases (Ph+) was 8% (0-100). The BCR-ABL landmarks of 1% and 10% after 3 months of imatinib both proved to discriminate significantly for PFS and OS: BCR-ABL IS <1% (n=233) vs. ≥1% (n=486), p=0.041 for PFS, p=0.048 for OS; BCR-ABL IS <10% (n=524) vs. ≥10% (n=195), p=0.004 for PFS and p=0.001 for OS. A stratification in 3 risk groups according to the achievement of a BCR-ABL IS of <1%, 1-10% and >10% after 3 months resulted in a significant difference between the poor risk group (>10%, n=195) and the intermediate risk group (1-10%, n=291): p=0.038 for PFS and p=0.012 for OS. The difference between the intermediate risk group and the good risk group (<1%, n=233) was not significant. The five year survival probability was 97%, 94% and 87% for the good, intermediate and poor risk group, respectively. Cytogenetic response landmarks after 3 months of imatinib were also predictive for PFS and OS: Ph+ ≤35% (n=362) vs. Ph+ >35% (n=123), p=0.022 for PFS, p=0.043 for OS; Ph+ ≤65% (n=401) vs. Ph+ >65% (n=84), p=0.004 for PFS and p=0.011 for OS. A 3 group stratification did not reach statistical significance. *Conclusions:* The achievement of molecular and cytogenetic response landmarks after 3 months of imatinib treatment is predictive for long term progression free and overall survival. At 3 months a BCR-ABL IS of 10% or more is associated with a 5-year overall survival of 87% suggesting an early change of treatment, whereas a BCR-ABL IS of 1% or less indicates a favorable 5-year overall survival of 97%.

6.4. **[784] Favorable Therapeutic Responses in Newly Diagnosed CML-CP Patients Induced by Dasatinib Are Reflected At the CD34+CD38+ Progenitor Cell but Not At the CD34+CD38- Stem Cell Level: Results From Randomized NordCML006 Study.** *Mustjoki.* Background: *In vitro* studies have suggested that CML stem cells are resistant to tyrosine kinase inhibitors (TKIs), but *in vivo* effects in patients have not been prospectively assessed. Furthermore, the inter-individual variation of the leukemic stem cell pool at diagnosis and its possible prognostic value is unknown. Patients: 46 newly diagnosed CML-CP patients were randomized 1:1 to receive either dasatinib 100 mg or imatinib 400 mg QD. The primary endpoint was a comparison of the proportion of Ph+ cells in CD34+CD38- and CD34+CD38+ compartment at 6 months between the study arms. Key secondary endpoints were the fraction of Ph+ cells in the stem cell compartments at 1 and 3 months, and molecular and cytogenetic responses at 3, 6, 12 and 18 months. Experimental endpoints included the percentage of Ph+ cells in the stem cell compartment at diagnosis and its correlation with therapeutic response. Results: One patient in the imatinib arm and none in the dasatinib arm progressed to blast crisis within first 12 months. 4/22 of dasatinib patients have discontinued the treatment due to side-effects (mainly pleural effusion) and 1 patient due to insufficient response. 3/24 imatinib patients have discontinued the therapy (1 blast crisis, 1 side-effects, 1 other malignancy).

Early cytogenetic responses were superior in the dasatinib arm: the median percentage of Ph+ cells in the bone marrow was 81% (imatinib) vs. 70% (dasatinib) at 1 month (p=0.15) and 5% vs. 0% at 3 months (p=0.0085). At 12 months all dasatinib (n=20) and 19/20 imatinib patients were in CCyR (results based on patients on treatment at 12 months). MMR rate was significantly higher in the dasatinib arm already at 6 months (70% vs. 20%, p=0.002) and similarly at 9 (75 vs. 26%, p=0.004) and 12 months (88% vs. 40%, p=0.009). Undetectable BCR-ABL1 transcripts (at least CMR4) were observed in 20% of the dasatinib patients at 6 months compared to none in the imatinib arm (p=0.11) and 44% in the dasatinib arm at 12 months compared to 7% in the imatinib arm (p=0.037). The median percentage of Ph+ cells, as measured by FISH (1000 cells analyzed), in the CD34+CD38- fraction at diagnosis was 79% (range 1-100%) compared to 96% (range 50-100%) in CD34+CD38+ fraction. The proportion of Ph+ cells in CD34+CD38- fraction correlated with WBC count (r=0.50, p<0.001), splenomegaly (r=0.43, p=0.0055), anemia (r=-0.44, p=0.004) and blood blast percentage (r=0.57, p=0.0001) at diagnosis. There was also a significant correlation between Ph+ cells in CD34+CD38- fraction at diagnosis and cytogenetic response at

1 (r=0.63, p<0.0001), 3 (r=0.48, p=0.0025) and 6 months (r=0.36, p=0.0271). Furthermore, leukemic stem cell burden at diagnosis correlated significantly with BCR-ABL1 transcript levels at 3 (r=0.54, p=0.0005), 6 (r=0.42, p=0.0088) and 9 months (r=0.40, p=0.0123). All patients who were not in MMR at 18 months, had >79% of Ph+ cells in CD34+CD38- fraction at diagnosis. During TKI therapy, the proportion of Ph^+ cells decreased rapidly in the stem cell fractions. At 1 month, the median proportion of Ph^+ cells was 14% and 56% in CD34+CD38- and CD34+CD38+ fractions compared to 69% in whole BM (p<0.0001, n=38). At 3 months, the respective numbers were 0.40%, 0.20% and 0.80% (p=0.087, n=33) and at 6 months 0%, 0% and 0.1% (p=0.23, n=41). Dasatinib-treated patients had significantly lower proportion of Ph+ cells in CD34+CD38+ fraction at 3 months than imatinib patients (0.05% vs. 0.68%, p=0.0318). A similar trend was also observed at 1 month (24% vs. 69%, p=0.05), but no difference existed at 6 months. In CD34+CD38- fraction the proportions of Ph+ cells did not differ significantly at 1 (11 vs. 17%, p=0.91), 3 (0.2 vs. 0.3%, p=0.44) or 6 months (0 vs. 0%, p=0.75). **Conclusions:** In comparison to imatinib, dasatinib induced superior therapeutic responses with remarkably high MMR and CMR rates. This was associated with a faster reduction of Ph+ cells at the progenitor CD34+CD38+ cell level. Surprisingly, both drugs rapidly depleted Ph+ cells from the more primitive CD34+CD38- compartment. The proportion of Ph^+ stem cells at diagnosis varied significantly among individual patients and bore prognostic value. Patients with a low proportion of Ph+ leukemic stem cells at diagnosis achieved faster and better cytogenetic and molecular responses. The leukemic stem cell burden at diagnosis may be a biomarker predicting treatment outcome and reflecting key biological factors in CML.

6.5. **[785] The Predictive Value of Early Molecular Response in Chronic Phase CML Patients Treated with Dasatinib First Line Therapy.** *Marin.* We assessed the correlation between molecular response at 3 and 6 months of dasatinib 100mg daily treatment and subsequent cytogenetic and molecular responses in 150 newly-diagnosed chronic phase CML patients treated with front line dasatinib in the UK SPIRIT 2 study (imatinib vs dasatinib). The median age was 54 years (range 18.4-82.1); 90 patients were male. The Sokal risk distribution was: 39 low, 65 intermediate and 46 high. At diagnosis 26 patients had splenomegaly >10cm below the costal margin; median WBC and platelet count were 65.7 (2.2-428) and 404 (101-2,433). The median hemoglobin level was 11.0 g/dl (4.17-15.8). The median percentage of blasts and basophils in peripheral blood was 0.4% (0-13.5) and 3.6% (0-19.2) respectively. The dose of dasatinib was adjusted according to tolerance. BCR-ABL1 transcripts in the peripheral blood were analyzed at 12 week intervals using RQ-PCR. Results were expressed as percentage ratios relative to an ABL1 internal control and expressed on the international scale. Complete molecular response (CMR) was defined as two consecutive samples with no detectable transcripts (RQ-PCR negative) and ABL1 control >40,000 (the median ABL1 control in the CMR samples was 96,000). In addition, we also explored a less stringent definition of CMR, namely CMR4.5 which was recently defined by the EUTOS group as BCR-ABL1 ratio of 0.0032 on the international scale, consistent with a 4.5 log reduction in the transcript level, without necessarily being RQ-PCR negative. With a median follow up of 15 months (range 6-29) the 2 year cumulative incidences (CI) of CCyR, MMR, CMR4.5 and CMR were 84.5, 72.1, 24.1 and 5.6% respectively. The median BCR-ABL/ABL ratios at 3, 6, 12 and 24 months were 0.830%, 0.093%, 0.040% and 0.034% respectively. We investigated the predictive value of the BCR-ABL1 transcript levels at 3 (>10% vs ≤10% and >1% vs ≤1%) and 6 months (>1% vs ≤1%) of dasatinib therapy on the 2 years CI of cytogenetic and molecular responses. The 135 patients who at 3 months had a BCR-ABL1/ABL1 ratio ≤10% and the 81 patients who had a ratio ≤1% had a significantly better 2 year CI of CCyR (89.1% vs 50.2%, p=0.02 and 100% vs 84.7%, p=0.01), MMR (83.7% vs 14.2%, p=0.004 and 85.2% vs 54.3 p<0.001), CMR4.5 (25.0% vs 0%, p<0.18 and 37.6 v 3.3% p=0.001) but not CMR (6.7 vs 0%, p=0.51 and 7.1 vs 0% p=0.46). Similarly, the 109 patients who at 6 months had a transcript ratio ≤1% had a better 2 year CI of MMR (86.3 vs 13.9%, p<0.001), CMR4.5 (31.2 vs 0%, p=0.03) and CMR (14.3 vs 0%, p=0.04) than the remaining patients. We used a receiver operating characteristic (ROC) curve to identify the optimal cut-off in the transcript level at 3 and 6 months that would predict the probability of each outcome with maximal sensitivity and specificity. Table 1 shows the results of applying the optimal cutoffs for each outcome in the 3-month analysis. Then we investigated whether the various outcomes could be better predicted using the cut-offs defined at 3 or at 6 months (including both the 1 and 10% cut-offs and the newly identified cut-offs) by using a multivariate model. For each outcome the cut-off defined at 3 months shown in table 1 was superior. No pre-therapy patient characteristics were an independent predictor for cytogenetic or molecular response. The key finding from this analysis is that patients who achieve a transcript level ≤10% after 3 months of dasatinib (135 of 150) have an 89.1% probability of eventually achieving CCyR, compared to 50.2% for patients with higher transcript levels (p=0.02). This preliminary observation may allow the identification of around 10% of dasatinib-treated patients for whom other forms of treatment might be considered although our conclusions require verification in further studies. The predictive power of RQ-PCR assessment can be greatly improved by identifying the optimal cut-offs for the specific outcomes, which is particularly important when predicting for the achievement of CMR. It remains uncertain whether these differences in response will translate into differences in survival and the SPIRIT 2 study continues to address this question.

6.6. **[786] Long Term Follow-up of Ph+ CML Patients Achieving Complete Cytogenetic Response (CCgR) with Interferon Based Therapy - GIMEMA Protocol CML0509.** *Russo* Interferon alpha (INFα) either alone or in combination with Ara-C was the frontline therapy of Ph+ chronic myeloid leukaemia (Ph+ CML) between 1980 and 2000. INFα prolonged survival as compared to conventional chemotherapy and it was the first drug able to induce complete cytogenetic responses (CCgRs). Patients achieving a CCgR by INFα ± Ara C were less than 10-15%, but they represent fascinating elite of patients who are the most likely candidates for prolonged survival and possibly for cure. The last update of the largest European cohort of 317 CML patients who had obtained a CCgR on IFNα was reported in 2001 (Bonifazi et al., Blood, 2001). Briefly, the median time to first CCgR was 19 months, the 10 year survival rate from first CCgR was 72% and the survival probability for patients with low Sokal risk was 84%. In this study, the contribution of the Italian Cooperative Study Group on CML was of 119 cases. Although INFα was used more than 20 years ago, obtaining information on this selected category of CCgR–INFα responding patients in the Imatinib era may be interesting both from the biological and clinical point of view. We report here the preliminary results of an observational study aimed to update the overall survival (OS), the progression free survival (PFS) to accelerated-blastic phase (ABP) and the CCgR duration in 92 Ph+ CML patients who were treated in Italy with an

INFα based therapy between 1986 and 2001 and who obtained a CCgR at least once . In this selected cohort of patients, the median time to first CCgR was 24 months (range, 3-143), and the median duration of the first CCgR was 87 months (3-252). The overall survival (OS) calculated from diagnosis, from start of IFNα and from the time of the first CCgR was 185 months (range, 74-334), 179 months (range, 74-287) and 154 months (range, 51-274), respectively. This is the longest follow-up of Ph+ CML patients who obtained a CCgR with an IFNα-based therapy. Out of 92 patients in CCgR, 71 (77%) cases stopped IFNα and 21 (23%) continued to be treated with IFNα. Out of the 71 cases who stopped IFNα, 38 (53%) cases lost CCgR and 3 (4%) cases died because of progression to ABP; 15 (21%) maintained CCgR without any other therapy and 18 (25%) maintained CCgR but shifted to Imatinib. Among the latter 33 patients maintaining the CCgR, 4 cases died because of CML unrelated causes. Out of the 21 cases who continued to be treated with IFNα, 18 (86%) currently maintain the CCgR and are alive and well, while 3 lost CCgR and died (2 cases for ABP). These data show that there are at least 33/92 (36%) patients who are alive and well and are maintaining a CCgR, either with continued IFNa treament (18 cases) or after IFNα treatment discontinuation (15 cases) but who have never been treated with Imatinib or any other tyrosine kinase inhibitor (TKI). We are now analyzing molecular data and we are collecting peripheral blood and bone marrow samples for correlative biological studies aimed to characterize the peculiar genetic and epigenetic features of these patients.

7 Biology and Pathophysiology: Extrinsic and Intrinsic Survival Mechanisms [961-966]

7.1. **[961] The Leukemic Stem Cell (LSC) in Ph^+ CML Is a $CD34^+/CD38^-/Lin^-$ Cell That Co-Expresses Dipeptidylpeptidase IV (CD26) and Disrupts LSC-Niche Interactions by Degrading the CXCR4 Ligand SDF-1α.** *Herrmann.* In Philadelphia-positive (Ph^+) chronic myeloid leukemia (CML), leukemic stem cells (LSC) supposedly reside in a $CD34^+/CD38^-/Lin^-$ fraction of the leukemic clone. However, little is known about phenotypic properties of LSC in CML. We screened for novel LSC markers and targets in CML by gene chip studies and extensive flow cytometry analyses using monoclonal antibodies against various surface antigens (n=50). A total number of 240 bone marrow or peripheral blood samples (CML, n=95; AML, n=103; CMML, n=10, control marrow, n=32) were examined. In common with normal SC, $CD34^+/CD38^-$ CML LSC were found to co-express the homing-receptor CD44, G-CSF-R (CD114), KIT (CD117), FLT3 (CD135), and CXCR4 (CD184). Similar to LSC in AML and CMML, CML LSC were found to display higher levels of Siglec-3 (CD33) and IL-3RA (CD123). Most significantly, however, we found that in contrast to normal $CD34^+/CD38^-$ stem cells, $CD34^+/CD38^-$ CML LSC aberrantly express IL-2RA (CD25), dipeptidylpeptidase IV (DPPIV=CD26), and IL-1RAP. In other myeloid leukemias (AML, CMML), $CD34^+/CD38^-$ LSC also co-expressed CD25, but usually did not express CD26 or IL-1RAP. Whereas CD26 was expressed almost invariably on $CD34^+/CD38^-$ cells in all CML patients tested, the surface enzyme was neither detectable in more mature $CD34^+/CD38^+$ progenitor cells nor on $CD34^+/CD38^-$ stem cells in reactive bone marrow or healthy controls. During successful treatment with imatinib or nilotinib (patients examined at CCyR and/or MMR), $CD34^+/CD38^-$ stem cells invariably showed a 'normal' phenotype ($CD25^-$, $CD26^-$, $IL\text{-}1RAP^-$), whereas in relapsing CML, $CD34^+/CD38^-$ cells were again found to co-express CD25 and CD26. Sorted $Lin^-/CD26^-$ stem cells obtained from CML patients (at diagnosis) engrafted irradiated NOD-SCID IL-2R$\gamma^{-/-}$ (NSG) mice with normal multilineage $BCR/ABL1^-$ hematopoiesis, whereas $Lin^-/CD26^+$ stem cells were found to engraft NSG mice with BCR/ABL^+ cells. We next examined the regulation of expression of CD25 and CD26 on CML LSC. Whereas expression of CD25 was found to depend on BCR/ABL1 and STAT5-activity, CD26 expression was found to be expressed independent of BCR/ABL1 and independent of STAT5-signaling. In a next step, we examined the potential function of CD26 on CML LSC. In these studies, CD26 was identified as a target-enzyme disrupting the niche-related SDF-1α/CXCR4 axis by degrading SDF-1α. Correspondingly, CD26-targeting gliptins (sitagliptin, 1 μM; vildagliptin, 1 μM) were found to revert recombinant DPPIV/CD26-induced or cellular CD26-induced inhibition of SDF-1α-mediated *in vitro* migration of $CD26^+$ leukemic cells. Finally, we found that in a CML patient treated with nilotinib, in whom uncontrolled diabetes mellitus required therapy with saxagliptin, BCR/ABL1 levels (in percent of ABL according to IS) that were found to increase before the start of saxagliptin (IS before saxagliptin: 1.6 [-4 months], 2.3 [-3 months], and 2.4 [at therapy-start]), decreased over time during saxagliptin-therapy (IS: 1.0 [+1 month], 1.0 [+3 months], 0.8 [+5 months]). Together, the CML-initiating LSC is a $CD34^+/CD38^-$ cell that exhibits aberrant expression of IL-1RAP, CD25, and DPPIV/CD26. All three markers may be useful for purification of CML LSC. DPPIV/CD26 appears to be a functionally and pathogenetically relevant antigen that may facilitate niche-independent uncontrolled redistribution and thus extramedullary spread of LSC and LSC-derived progenitor cells in CML. Whether CD26 can be developed as a novel therapeutic target in CML is currently under investigation.

7.2. **[962] Leukemia-Induced Alterations in Bone Marrow Cytokine and Chemokine Levels Contribute to Altered Stem Cell Lodgment and Impairment of Normal Stem Cell Growth in CML.** *Zhang.* Specialized bone marrow (BM) microenvironmental niches are essential for hematopoietic stem cell (HSC) lodgment and maintenance. However microenvironmental interactions of leukemia stem cells (LSC) are poorly understood. Although chronic myelogenous leukemia (CML) results from HSC transformation by the BCR-ABL gene, the role of the microenvironment in modulating leukemia development is not known. We employed the SCL-tTA-BCR/ABL mouse model of CML to investigate the LSC interactions with the BM microenvironment. In this model, targeted expression of the BCR-ABL gene in murine HSC via a tet-regulated SCL promoter results in development of a chronic phase CML-like disorder. We have reported that LSC capacity is restricted to BCR-ABL+ cells with long-term hematopoietic stem cell (LTHSC) phenotype(LSK Flt3-CD150+CD48-) (Blood 2010 116:1212A). LSC numbers are reduced in the BM but increased in the spleen of CML mice compared with LTHSC from control mice, suggesting that LSC have altered niche interactions. LSC also demonstrate altered trafficking with significant reduction in homing of IV injected LSC to BM, and markedly increased egress of intrafemorally injected LSC to the spleen, potentially related to reduced CXCL12 levels in the BM of CML mice. In addition, levels of several chemokines and cytokines, including MIP1α, MIP1β, MIP2, IL-1α, IL-1β, TNF-α, G-CSF and IL-6, were increased in CML BM, related to increased production by malignant hematopoietic cells. We investigated whether altered chemokine and cytokine expression was associated with altered capacity of the CML BM microenvironment to support

LTHSC engraftment. LTHSC from control mice or LSC from CML mice were transplanted into irradiated CML or control recipients. There was reduced engraftment of both control LTHSC and CML LSC in the BM of CML compared to control recipients at 2 weeks after transplantation, associated with reduced homing to CML BM, potentially related to low BM CXCL12 levels. The numbers of control LTHSC in the BM of CML recipient mice remained low at 4 weeks post-transplantation, whereas the numbers of CML LSC increased to numbers similar to those seen in the BM of control recipients. Culture with CML BM supernatants (SN) resulted in impaired growth of control LTHSC compared to control BM SN. In contrast the growth of CML LSC was similar following culture with CML and control BM SN. Culture with individual factors at concentrations similar to those observed in CML BM (16ng/ml MIP1α, 8ng/ml MIP1β, 2.5ng/ml IL-1α, 3.5ng/ml IL-1β, 0.05ng/ml TNF-α) resulted in significantly reduced growth of normal LTHSC compared with CML LSC. These results indicate that diffusible factors produced by leukemic cells in the CML BM environment selectively inhibit normal LTHSC compared to CML LSC growth. Exposure of a murine stromal cell line to CML BM SN resulted in reduced CXCL12 mRNA levels compared to BM SN from control mice. The cytokine G-CSF, which was increased in CML BM SN, has been reported to reduce CXCL12 transcription. We observed significant reduction of CXCL12 mRNA levels in stromal cells cultured with G-CSF (0.2ng/ml), supporting a potential role for increased G-CSF production by leukemia cells in reduced CXCL12 production by CML BM stromal cells and reduced LSC retention in the BM. We evaluated whether defects in microenvironmental function in CML were affected by imatinib treatment. Treatment of CML mice with imatinib (200mg/kg/day, 2 weeks) led to reduction in MIP1α, MIP1β, IL-1β, and IL-6 levels in BM cells. Engraftment of normal LTHSC was significantly enhanced in BM of CML recipients pre-treated with imatinib. Results obtained with the mouse model were validated using specimens obtained from CML patients. CXCL12 mRNA levels were significantly reduced in human CML compared to normal MNCs, whereas expression of MIP1α, MIP-2, IL-1α and IL-1β were increased in CML MNCs, consistent with results obtained with the mouse model. Coculture with CML MNC conditioned medium (CM) resulted in selective impairment of growth of normal CD34+CD38- primitive progenitors compared to CM from normal MNC, but did not inhibit growth of CML progenitors. We conclude that leukemia-induced alterations in BM cytokine and chemokine levels contribute to altered LSC lodgment and to selective impairment of growth of normal LTHSC in the CML BM microenvironment, leading to a relative growth advantage for CML LSC over normal LTHSC and expansion of the leukemic clone.

7.3. **[963] Targeting of a Novel MNK-eIF4E-b-Catenin Axis in Blast Crisis Chronic Myelogenous Leukemia Inhibits Leukemia Stem Cell Function.** *Lim.* Blast crisis (BC) chronic myeloid leukemia (CML) is characterized by expansion of a granulocyte macrophage progenitor-like population (GMPs) that has acquired self-renewal capacity, a feature not seen in normal or chronic phase (CP) GMPs. The ability to self-renew is thought to be mediated by b-catenin activation, and may contribute to disease persistence, as well as act as a reservoir for resistance. The mechanisms contributing to b-catenin activation remain obscure, and will need to be identified to improve the control of BC. In this study, we investigated the role of the mRNA translation machinery in mediating b-catenin-mediated self-renewal, since our prior work had implicated aberrant mRNA translation in drug-resistance and BC pathophysiology (Ly et al. Cancer Research 2003; Prabhu et al. Oncogene, 2007; Zhang et al. MCB, 2008). Using immunofluorescence (IF), we first confirmed that BC GMPs have activated nuclear b-catenin compared to GMPs isolated from normal cord blood, and that this was associated with increased eIF4E expression and phosphorylation at Ser209. Next, using biochemical and genetic approaches in CML cell lines (K562 and KCL22), we demonstrated that eIF4E overexpression was sufficient to increase b-catenin activity (as measured by IF for nuclear b-catenin, b-catenin reporter assays, and expression of b-catenin-regulated genes). By expressing phospho-mutant forms of eIF4E (S209A, S209D), we also found that the increase in b-catenin transcriptional activity is dependent on phosphorylation of at Ser209. In line with these observations, siRNA-mediated knockdown or pharmacologic (CGP57380) inhibition of the MNK1/2 kinases (which mediate *in vivo* eIF4E phosphorylation) prevented the increased b-catenin activity induced by eIF4E overexpression. Mechanistically, we found that eIF4E activated b-catenin signaling via a two-step mechanism. First, eIF4E overexpression increased total cell b-catenin. Second, eIF4E phosphorylation facilitated b-catenin nuclear translocation. The latter step was associated with increased b-catenin phosphorylation at Ser552, a site known to be involved in nuclear translocation, and directly regulated by AKT. Consistent with this model, siRNA-mediated knockdown or small molecule inhibition of AKT (AKT Inhibitor IV) prevented eIF4E-mediated increases in b-catenin transcriptional activity. The importance of eIF4E phosphorylation on b-catenin activation and the self-renewal capacity of primary BC GMPs cells was assessed next. First, we showed that treatment with CGP57380, but not imatinib or dasatinib, inhibited eIF4E phosphorylation, as well as prevented accumulation of active nuclear b-catenin in BC GMPs. Next, we evaluated the effect of MNK1/2 inhibition on the stem cell function of BC cells using both *in vitro* and *in vivo* assays. In an *in vitro* serial replating assay, we showed that CGP57380 impaired the ability of CD34+ BC cells (including those carrying T315I mutation), but not normal CD34+ cells, to serially replate for more than 8 weeks in methylcellulose. Interestingly, treatment with either imatinib or dasatinib only partially impaired the ability of BC CML to serially replate. Next, we found that *in vitro* treatment of BC CD34+ CML cells, but not normal cord blood CD34+ cells, with CGP57380 retarded their ability to engraft NSG mice. Finally, we developed an *in vivo* serial transplantation assay for assessing the leukemia stem cell (LSC) function of patient-derived BC GMPs. Here, we injected either BC GMPs or BC CD34+ CML cells intrafemorally into 8- to 10-week old sublethally irradiated NSG mice. Following engraftment, mice were treated with vehicle, CGP53780 (40 mg/kg/d), or dasatinib (5mg/kg/d) for three consecutive weeks. Following treatment, human CD34+ cells were isolated from the mice, and transplanted into a second recipient mouse. At 16 weeks, we found that *in vivo* treatment with CGP57380, but not dasatinib, prevented BC cells from serially transplanting NSG mice. In summary, our results demonstrate that: 1. eIF4E is overexpressed and phosphorylated at Ser209 in BC, but not normal, GMPs; 2. eIF4E phosphorylation activates b-catenin signalling in BC GMPs; 3. MNK inhibition prevents eIF4E phosphorylation and b-catenin signalling in BC GMPs; and 4. MNK inhibition prevents BC GMPs from functioning as LSCs. Our studies suggest that pharmacologic inhibition of the MNK1/2 kinases may be therapeutically useful in BC CML.

7.4. [964] Regulation of Chronic Myelogenous Leukemia Stem Cells by Leukemia Oncogene Evi1. *Sato.* Chronic myelogenous leukemia (CML) is a hematopoietic stem cell (HSC) disease caused by BCR-ABL oncogene, and now newly

targeted therapies are warrented for CML due to the minimal effect of BCR-ABL-targeted tyrosine kinase inhibitors toward CML stem cells. As CML stem cells are known to show some similarity with HSC, utilizing HSC-specific factors as a guide for analyzing CML stem cells is of great significance. Since Evi1 is a transcription factor which is highly expressed within normal HSC compartment and it is frequently activated in myeloid malignancies including a blastic phase (blast crisis) of CML (CML-BC), it is supposed that CML stem cells could have a close relation with Evi1. Here in this study, with Evi1-GFP knock-in mice, which we have recently generated, we developed murine models of CML in a chronic phase (CML-CP) and CML-BC for uncovering new properties of CML stem cells. In Evi1-GFP knock-in CML-CP model, we found that Evi1 positive CML cells account for about 0.1-0.5% of total bone marrow (BM) cells and that almost all of them showed no lineage markers. Furthermore, Evi1 is predominantly expressed in the CML stem cell fraction (Lin- Sca-1+ c-kit+ (LSK)), but its expression is sharply downregulated even in myeloid progenitor (Lin- Sca1- c-kit+ (MP)) cells and in more differentiated cells. Even within CML LSK cells, Evi1 expression widely varies and Evi1-high LSK cells show an enhanced colony-forming capacity compared with Evi1-low LSK cells. As for cell cycle status, Evi1-high CML LSK cells are mostly in G0/G1 phase although Evi1-low CML LSK cells or CML myeloid progenitor are more in S/G2/M phase. When CML LSK cells are cocultured with OP-9 stromal cells, only Evi1-high LSK cells could made cobblestone areas. Comparison of Evi1-high cells with Evi1-low cells in normal and CML LSK compartments by gene expression profiles showed that a more quiescent feature and a less differentiated feature in Evi-high CML LSK cells than in Evi1-low CML LSK cells. Moreover, Evi1-high CML LSK cells have a close correlation with TGF-beta signaling and calcium signaling. In addition, Evi1-high normal LSK cells had the most quiescent and the least differentiated profiles, which suggested that Evi1-high CML LSK cells could keep self-renewal capacity with high proliferation capacity. In concert with our data of Evi1-trafficking CML mouse, in CML patients, we have also recently found that CD34+ 38- CML stem cells showed higher EVI1 expression than CD34+ 38+ CML progenitor cells or total CML cells, which implies that EVI1 could mark CML stem cells as well as normal HSCs. These data indicate that in our Evi1 trafficking CML model high Evi1 expression could enrich CML stem cells and that Evi1 could have a crucial role in CML pathogenesis. In Evi1-GFP knock-in CML-BC model, which more differentiated myeloid progenitors are likely to have a high leukemia initiating potential, a sizable fraction of MP leukemic cells show distinct Evi1 expression. Remarkably, in vivo transplantation assay revealed CML-BC stem cells that can recapitulate the disease are exclusively enriched in Evi1-high MP fraction. Evi1-high MP cells showed a replating capacity in colony assay while Evi1-low MP cells could not. Moreover, Evi1-high MP cells are more actively cycling than Evi1-low MP cells. Our data revealed a limited fraction with high Evi1 expression within stem/progenitor cells possesses enhanced proliferative and leukemia-initiating capacities in CML. As opposed to these CML models noted above, in Evi1-GFP knock-in AML model by MLL-ENL, Evi1-high leukemic cells showed no advantage in leukemia initiating potential. Additionally, other Evi1-GFP knock-in AML models by MOZ-TIF2 and TEL-PDGFRb/AML1-ETO never showed Evi1-high fraction both in BM and spleen, which might suggest the high affinity of Evi1 with stem cell disease as CML. The current study provides us with a new tool for dissecting pathogenesis and exploiting novel targeted therapies to eradicate CML stem cells. An establishment of Evi1-related therapy for CML stem cells, which could be applied to EVI1-high malignancies, is currently being explored.

7.5. **[965] Without GABP Transcription Factor, BCR-ABL Cannot Transform HSCs to Leukemic Stem Cells Nor Induce Chronic Myelogenous Leukemia in Mice.** *Yang.* Chronic Myelogenous Leukemia (CML) is driven by the fusion oncogene, BCR-ABL, which transforms normal hematopoietic stem cells (HSCs) to leukemic stem cells (LSCs). Tyrosine kinase inhibitors, such as imatinib mesylate, control the massive expansion of leukemic cells in most patients with CML, but cannot eradicate CML LSCs. Several genetic pathways have been shown to be critical for the growth and survival of CML LSCs, including signaling molecules, tumor suppressors, and metabolic regulators. However, the role of transcription factors in functional regulation of LSCs in CML has not been widely studied. GA Binding Protein (GABP) is an ets transcription factor that is required for entry of fibroblasts into the cell cycle, and expression of Gabpa (the DNA-binding component of the complex), alone, was sufficient to induce quiescent, serum-starved cells to enter the cell cycle. Thus, Gabp is both necessary and sufficient for cell cycle entry. Conditional deletion of Gabpa in mouse bone marrow decreased hematopoietic progenitor cells more than 100-fold, but hematopoietic stem cells (HSCs) were relatively preserved. Gabpα null HSCs exhibited significant cell cycle arrest. We sought to determine if the cell cycle arrest caused by Gabpa loss could impair development of CML cells in a mouse model. We used retroviral infection of bone marrow from 5-FU-treated mice (to enrich for stem and progenitor cells) to generate a rapidly fatal CML-like syndrome in mice. Bone marrow from mice with loxP-flanked (floxed) Gabpa and wild type control mice was infected with a retrovirus that co-expresses BCR-ABL, Cre recombinase, and green fluorescent protein (GFP). As expected, transplantation into recipient mice of control mouse bone marrow infected with BCR-ABL-Cre-GFP retrovirus caused a rapidly fatal myeloproliferative neoplasm, with a median survival of approximately three weeks; mice died with massive infiltration of GFP+ myeloid cells in peripheral blood cell, spleen, bone marrow, and other organs. In floxed Gabpa bone marrow, the retrovirus deleted floxed Gabpa in cells that express the BCR-ABL fusion oncogene, and these cells were identifiable based on GFP expression. Transplantation of floxed Gabpa bone marrow infected with BCR-ABL-Cre-GFP retrovirus failed to induce CML during six months of observation. Importantly, GFP+ peripheral blood granulocytes were observed for at least 6 months after transplantation; these CD11b+, Gr1+ cells continued to express BCR-ABL and were shown to be Gabpa null. These results indicate that the lack of Gabpa severely impaired the function of LSCs. In addition, secondary transplantation of bone marrow from these mice again demonstrated the presence of BCR-ABL-expressing peripheral blood myeloid cells. We conclude that Gabp transcription factor is required for the transformation of HSCs to LSCs by BCR-ABL. Furthermore, the persistence of BCR-ABL-expressing myeloid cells without the development of leukemia provides a unique model that permits analysis of the biological properties of BCR-ABL in vivo. The continued generation of BCR-ABL-expressing cells without CML development is unprecedented, and represents a unique model of leukemia tumor suppression.

7.6. **[966] Molecular and Structural Characterization of the SH3 Domain of AHI-1 in Regulation of Cellular Resistance of BCR-ABL$^+$ Chronic Myeloid Leukemia Cells to Tyrosine Kinase Inhibitors.** *Liu.* Chronic myeloid

leukemia (CML) is a clonal multilineage myeloproliferative disorder characterized by the presence of the fusion gene *BCR-ABL* with increased tyrosine kinase activity. Imatinib mesylate (IM) and other BCR-ABL tyrosine kinase inhibitors (TKIs), including dasatinib (DA) and nilotinib (NL), have been introduced into clinical practice with remarkable effects on chronic phase CML. However, early relapses, acquired drug resistance, and persistence of leukemic stem cells remain problematic. Improved treatment approaches to target other key molecular elements active in CML stem/progenitor cells are needed. One candidate is *AHI-1* (Abelson helper integration site 1), an oncogene that is highly deregulated in CML leukemic stem cells. It harbors two key domains, SH3 and WD40-repeat, which are known important mediators of protein-protein interactions. We recently demonstrated that AHI-1 physically interacts with BCR-ABL and JAK2 in CML cells and this interaction complex mediates transforming activity and TKI response/resistance of CML stem/progenitor cells. We have also shown that AHI-1 interacts independently with JAK2 and BCR-ABL via different binding sites to mediate their activities. In this study, we have characterized the biological and structural functions of the SH3 domain of AHI-1. To determine roles of the SH3 domain in regulation of cell proliferation and TKI response/resistance, several mutant forms, including SH3 domain deletion ($SH3^{\Delta}$), double WD40-repeat and SH3 domain deletion ($SH3WD40^{\Delta}$) and N-terminal deletion ($N\text{-}ter^{\Delta}$, containing SH3 and WD40-repeat domains) were generated and stably transduced into *BCR-ABL* inducible BaF3 cells, in which the level of expression of *BCR-ABL* can be down-regulated by exposure to doxycycline. Overexpression of full-length *Ahi-1* in *BCR-ABL* inducible cells resulted in fewer Annexin V+ apoptotic cells with doxycyclin (suppression of *BCR-ABL*) compared to *BCR-ABL* inducible cells (3 and 29% *v.s.*10 and 60% after 24 or 48 hours). Cells expressing the $SH3^{\Delta}$ mutant and the $SH3WD40^{D}$ mutant displayed dramatically increased Annexin V+ cells (10, 77% and 34, 90% *v.s.*3 and 29%), while cells expressing the $N\text{-}ter^{\Delta}$ mutant had similar numbers of Annexin V+ cells compared to *BCR-ABL* inducible cells (6 and 41% *v.s.*10 and 60%). Similarly, $BCR\text{-}ABL^{+}$ cells transduced with $SH3^{\Delta}$ and $SH3WD40^{D}$ mutants displayed significantly increased apoptotic cells compared to cells transduced with full-length *Ahi-1* in the presence of 2 μM IM (57, 87 vs. 26%), 2μM NL (65, 87 vs. 25%) and 150 nM DA (63, 96 vs. 34%) after 24 hour treatment. $BCR\text{-}ABL^{+}$ cells transduced with the $N\text{-}ter^{\Delta}$ mutant also showed more sensitivity to the drug treatments compared to the cells with the full-length *Ahi-1*(36 % for IM, 40% for NL and 40% for DA), but with lower sensitivity than cells carrying the *Ahi-1* SH3 domain deletion mutants, indicating that the SH3 domain of *Ahi-1* plays a role in the mediation of TKI resistance. The crystal structure of the AHI-1 SH3 domain at 1.32-Å resolution revealed that the AHI-1SH3 domain adopts a canonical SH3 folding, but with an unusual C-terminal α helix. There are three large negatively charged patches, which are constructed by the n-Src loop, the end of the RT loop and the C-terminal helix, and this special feature may be involved in binding selectivity and specificity. PD1R peptide, known to interact with the PI3K SH3 domain, was used to model the binding pattern between AHI-1 SH3 domain and its ligands, and there may be formation of an "Arg-Arg-Trp" stack within the binding interface, which could be a targeting site for designing specific drugs. Moreover, using the AHI-1 SH3 domain as protein 'bait' in immunoprecipitation/mass spectrometry, Dynamin-2 was identified as a potential interacting partner of AHI-1; both AHI-1 and Dynamin-2 are involved in trafficking and signaling processes. In conclusion, the investigation of the structure of AHI-1 SH3 domain and its interacting proteins will thus provide invaluable insight in identification of key interaction sites in regulation of drug resistance and may be utilized for development of small molecule inhibitors for CML.

8 Posters: Biology and Pathophysiology, excluding Therapy: Poster I [1666-1679]

1666. A Common Deletion Polymorphism in the *BIM* Gene Contributes to Intrinsic Imatinib Resistance in Chronic Myelogenous Leukemia
1667. Role of MicroRNA-486-5p Overexpression In CML CD34+ Cells In Modulating BCR-ABL Mediated Hematopoietic Stem/Progenitor Cell Transformation and Imatinib Sensitivity
1668. Loss of Stress Sensor GADD45a Accelerates BCR-ABL-Driven Leukemogenesis
1669. RNAi-Mediated Inhibition of Mcl-1 Expression Enhances Apoptosis in Imatinib-Treated CML Progenitors
1670. Parathyroid Hormone-Induced Modulation of the Bone Marrow Microenvironment Reduces Leukemic Stem Cells in Murine Chronic Myelogenous-Leukemia-Like Disease Via a TGFbeta-Dependent Pathway
1671. Loss of EGR-1 Accelerates BCR-ABL-Driven Leukemogenesis
1672. The Role of c-Abl in Jak2 Activation in IL-3-Dependent Cells
1673. Rewiring of the Protein Kinase C Beta 2 (PKC βII) and Bcr/Abl Signal Transduction Pathways
1674. Nilotinib, in Comparison to Both Dasatinib and Imatinib, Possesses a Greatly Prolonged Residence Time When Bound to the BCR-ABL Kinase SH1 Domain
1675. Differential Lineage Involvement Between Very Low and Higher OCT-1 Activity Chronic-Phase CML Patients
1676. C22*orf2* Gene (22q13.1) Translocation Downstream of Bcr Breakpoint (22q11) to the Derivative 9 Chromosome Is Associated with Reduced Expression of Its Protein, the Beta-Catenin Antagonist Chibby, in Chronic Myeloid Leukemia Progenitors
1677. A Cytotoxic Human Monoclonal Antibody Recognizing Cell Surface WT1 Peptide/HLA-A2 Complexes
1678. Leukemia Microenvironment-Specific Galectin-3 Expression of Leukemic Cells Promotes Malignant Niche Formation and Bone Marrow Lodgment of Leukemic Cells in Chronic Myelogenous Leukemia
1679. Absolute Quantification of Mutant T315I BCR-ABL Transcripts At 6 Months Identifies Imatinib Resistant CML Patients with a Low Probability to Achieve MMR After 12 Months on Second Line Tyrosine Kinase Therapy

9 Posters: Therapy: Poster I [1680-1702]

1680. Assessment of BCR-ABL Transcript Levels At 3 Months Is the Only Requirement for Predicting Outcome for Patients with Chronic Myeloid Leukemia Treated with Imatinib
1681. Time-Related Interpretation of Molecular Response Levels According to Long Term Overall and Progression-Free Survival of CML Patients on First-Line Imatinib Treatment
1682. Intermittent Imatinib (INTERIM) Treatment of Patients with Ph+ Chronic Myeloid Leukemia in Complete Cytogenetic Response: Cytogenetic and Molecular Data At One Year
1683. Clinical Significance of Additional Chromosomal Abnormalities In Ph-Positive and Ph-Negative Cells In Patients with Chronic Myeloid Leukemia Treated by Tyrosine Kinase Inhibitors
1684. The Month Three Major Molecular Response in Chronic Phase Chronic Myeloid Leukemia on $imatinib^{400}$, Nilotinib and Dasatinib Is a Major Prognostic Factor for Failure-Free and Progression-Free Survival.

1685. Safety and Management of Toxicities in the BELA Trial of Bosutinib Versus Imatinib in Newly Diagnosed Chronic Phase Chronic Myeloid Leukemia
1686/ APPLICATION of EUTOS SCORE IN CHRONIC Myeloid LEUKEMIA AFFECTING VERY Elderly (>75 years) PATIENTS
1687. Chronic Myeloid Leukemia: Molecular Monitoring of Residual Disease by Genomic DNA Compared to Conventional mRNA Analysis in Follow-Ups up to 8 Years
1688. Outcome of Patients with Chronic Myeloid Leukemia After Allogeneic Stem Cell Transplantation in Europe; Data From the EUTOS for CML Registry
1689. Effect of Time to Dasatinib Initiation On Outcome of Imatinib-Intolerant Patients with Chronic-Phase Chronic Myelogenous Leukemia (CP-CML): Results From a European Observational Study (FORTE; CA180-211)
1690. The Strategy of Early Nilotinib Switch Based on Failure to Achieve Optimal Molecular Targets on Imatinib May Not Overcome the Negative Impact of a Low OCT-1 Activity in De-Novo CP-CML Patients
1691. Shorter Time to Achieve a Major Molecular Response (MMR) Is Predictive of a Faster Complete Molecular Response in CML: A Study of Characteristics and Outcomes of a Cohort of CML Patients in MMR
1692. Multidrug Resistance Gene (MDR1) C3435T Polymorphism and Imatinib Response in Patients with Chronic Myeloid Leukemia
1693. Combination Targeted Therapy to Impair Self-Renewal Capacity of Human Blast Crisis Leukemia Stem Cells
1694. Clonal Evolution but Not Variant Chromosomal Translocations Is An Adverse Prognostic Marker for Cytogenetic Response and Survival in CML Patients Treated with Imatinib
1695. Survey of the Frontline Treatment and Management of Chronic Myeloid Leukemia (CML) in a Real-Word Setting: The 3rd Annual Update of the Worldwide Observational Registry Collecting Longitudinal Data on Management of Chronic Myeloid Leukemia Patients (The WORLD CML Registry)
1696. Clonal Dasatinib Large Granular Expansion Is Associated with Suboptimal and Optimal Leukemia Net Response Criteria in Chronic Myelogenous Leukemia
1697. Increased NK Cells and Decreased $CD3^+CD8^+CD62L^+$ T Cells in CML Patients Who Sustained Complete Molecular Remission After Discontinuation of Imatinib
1698. Comparison of Adverse Drug Reactions for Patients Treated with Tyrosine Kinase Inhibitors: Data Mining of the Public Version of the FDA Adverse Event Reporting System (AERS)
1699. Tyrosine Kinase Inhibitor Therapy Induced Changes in Humoral Immunity in Patients with Chronic Myeloid Leukemia
1700. Results of a Phase II Trial of Dasatinib As Frontline Therapy for Chronic Myeloid Leukemia (CML) In Chronic Phase (CP)
1701. Bcr-Abl Mutations in Chronic Myeloid Leukemia - Impact on Survival and Treatment with Second Generation Inhibitors– A Study on Behalf of Latin American Leukemia Net (Lalnet)
1702. Landmark Analysis of Imatinib Treatment in CML Chronic Phase: ES-FISH <10% Ph+ At 3 Months Associated with Better Cytogenetic Response and Improved Long-Term Event-Free Survival

10 Posters: Biology and Pathophysiology, excluding Therapy: Poster II [2735-2748]

2735. BCL2 Splice Isoform Switching Promotes Leukemia Stem Cell Survival and Sensitivity to a Novel Pan BCL2 Inhibitor
2736. Targeting Rac2 - Mitochondrial Respiratory Chain Complex III Signaling to Prevent Genomic Instability in Leukemia Stem and Progenitor Cells
2737Potentiation of the Effects of Nilotinib by Combination with Plerixafor in a Mouse Model of BCR-ABL-Positive Residual Disease
2738. Combined Pharmacologic Inhibition of Bcl-Xl/Bcl-2 and mTORC1/2 Survival Signals Trigger Apoptosis in BCR-$ABL1^+$ *in Vitro* Models of Blast Crisis Chronic Myelogenous Leukemia (CML-BC), and Primary $CD34^+/CD38^-$ Stem and $CD34^+$ progenitor Cells From CML-BC Patients
2739. CML Patients Present Additional Mutations in Cancer Related Genes When Tested At Diagnosis
2740. BCR-ABL Regulates Death Receptor Expression for TNF-Related Apoptosis-Inducing Ligand (TRAIL) in Philadelphia Chromosome-Positive Leukemia
2741. The Pro-Metastasis Tyrosine Phosphatase, PRL-3 (*PTP4A3*), Is a Novel Target of BCR-ABL Signaling Involved in Human Chronic Myeloid Leukemia
2742. Statins Increase Antileukemic Potency of Imatinib Through the Inhibition of MDR/ABCB1 and BCRP/ABCG2 Drug Transporters Activity
2743. Targeting the CML Stem/Progenitor Cell with JAK2 (BMS-911543)/ABL Inhibitor Combination Therapy
2744. Increased Levels of Myeloid-Derived Suppressor Cells (MDSCs) in Chronic Myeloid Leukemia and the Effect of Tyrosine Kinase Inhibitors on MDSCs *in Vitro*
2745. ATP Dependent Efflux Transporters ABCB1 and ABCG2 Are Unlikely to Impact the Efficacy, or Mediate Resistance to the Tyrosine Kinase Inhibitor, Ponatinib.
2746. Fingolimod (FTY720) Inhibits BCR-ABL Signaling Allosterically by Binding to the Myristate Binding Site
2747. Flow Cytometry Detection of Intra-Cellular Tyrosine Kinase Inhibitors (TKI) Showed Variable Uptake in CML CD34+ Cells
2748. The Imatinib and Nilotinib Induced Modulation of the Proteasomal Activity and Antigen Processing in Chronic Myeloid Leukemia Cells

11 Posters: Therapy: Poster II [2749-2771]

2749. Very Long-Term Follow-up Results of Imatinib Mesylate Therapy in Chronic Phase Chronic Myeloid Leukemia After Failure of Interferon Alpha Therapy
2750. Improved Survival in Chronic Myeloid Leukemia (CML) Since the Introductin of Imatinib Therapy - A Single Institution Historical Experience
2751. Age Influences Initial Dose and Compliance to Imatinib In Chronic Myeloid Leukemia Elederly Patients but Concomitant Comorbidities Appear to Influence Overall and Event-Free Survival
2752. Rapid and Sustained Increase of LGL and Rare CMV-Reactivation During Dasatinib Treatments in CML
2753. Nilotinib Exerts Direct Effects on Vascular Endothelial Cells and May Act As a Co-Trigger of Atherosclerosis in Patients with Ph+ CML
2754. Comparison of Adherence Between Nilotinib and Dasatinib As Second-Line Therapies in Chronic Myeloid Leukemia
2755. The Incidence of BCR-ABL Mutations and Their Impact on Outcome in Patients with Newly Diagnosed Chronic Myeloid Leukemia in Chronic Phase (CML-CP) Treated with Nilotinib or Imatinib in ENESTnd: 36-Month Follow-up
2756. Early CP CML, Nilotinib 400 mg Twice Daily Frontline: Beyond 3 Years, Results Remain Excellent and Stable (A GIMEMA CML Working Party Trial)

2757. Retrospective Cohort Analysis of Peripheral Arterial Occlusive Disease (PAOD) Events in Patients (pts) with Chronic Myeloid Leukemia in Chronic Phase (CML-CP)
2758. Preliminary Results From a Phase III Trial of Imatinib Versus Imatinib in Combination with Cytarabine in Patients with First Chronic Phase Myeloid Leukemia
2759. A Phase IIIb Multicentre Open-Label Study of Nilotinib in Adult Patients with Newly Diagnosed BCR-ABL Positive Chronic Myeloid Leukemia (CML) in Chronic Phase (CP): A European Clinical Initiative with EUTOS Collaboration for Standardisation of Molecular Remission
2760. Bosutinib Safety Profile and Management of Toxicities in Leukemia Patients with Resistance or Intolerance to Imatinib and Other Tyrosine Kinase Inhibitors
2761. Clinical Significance of Myelosuppression Associated with the Use of Dasatinib and Nilotinib As Initial Therapy in Chronic Phase (CP) of Chronic Myeloid Leukemia (CML)
2762. Impact of Tyrosine Kinase Inhibitor (TKI) in Chronic Myeloid Leukemia (CML) Relapsing After T Cell Depleted Allogeneic Stem Cell Transplantation (SCT)
2763. Discontinuation of Imatinib Therapy in Chronic Myeloid Leukemia Patients with Sustained Complete Molecular Response$^{4.5}$ (CMR$^{4.5}$)
2764. Safety and Efficacy of Frontline Nilotinib (Nb) for Chronic Phase (CP) Chronic Myeloid Leukemia (CML) in Diabetic Patients (pts)
2765. Efficacy of Nilotinib Versus High-Dose Imatinib in Early Chronic Phase CML Patients Who Have Suboptimal Molecular Responses to Standard-Dose Imatinib (RE-NICE Multicenter Study)
2766. Correlation of Molecular Response At 18 Month with Survival Benefits As Response-Related Prognostic Factor
2767. Dasatinib and Imatinib-Induced Reductions in BCR-ABL Transcript Levels Below 10% At 3 Months Are Associated with Improved Responses in Patients with Newly Diagnosed Chronic Myeloid Leukemia in Chronic Phase (CML-CP): Analysis of Molecular Response Kinetics in the DASISION Trial
2768. Impact of Dose Reductions and Interruptions Due to Adverse Events (AEs)on Efficacy in Newly Diagnosed Chronic Myeloid Leukemia in ChronicPhase (CML-CP) Patients (pts) Receiving Either Dasatinib (D) or Imatinib(IM): Analysis of the DASISION Trial
2769. The Incidence of Bcr-Abl Mutations and Their Impact on Clinical Outcome in Newly Diagnosed Chronic Phase Chronic Myeloid Leukemia Patients Treated with Bosutinib Versus Imatinib in the BELA Trial
2770. Imatinib in Very Elderly (> 75 years) CML Patients: Are Low-Doses (<400 mg daily) Enough?
2771. Effect of Nilotinib (NIL) on Molecular Response in Chronic Myelogenous Leukemia - Chronic Phase (CML-CP) Patients (pts) with a Suboptimal Molecular Response to Imatinib (IM) – ENABL Study Update.

12 Posters: Biology and Pathophysiology, excluding Therapy: Poster III [3741-3758]

3741. Side Effects on the Skeletal System Exerted by Continuous and by Interval Treatment with Tyrosine Kinase Inhibitors in An Animal Model of Juvenile Rodents
3742. Intrinsic and Extrinsic Survival Signals Converge on STAT3 As a Critical Mediator of BCR-ABL-Independent Tyrosine Kinase Inhibitor Resistance
3743. Socs2 Is Dispensable for BCR/ABL1-Induced Chronic Myeloid Leukemia-Like Disease in Mice and for Normal Hematopoietic Stem Cell Function
3744. Frequency and Clonality of BCR-ABL Compound Mutations in Chronic Myeloid Leukemia
3745. Antibody-Targeting of IL-3 Receptor-α Increases the Susceptibility of CD34^{+} CML Progenitors to Dasatinib-Induced Cell Death
3746. Galectin-3 Is the Molecular Target for Overcoming Multidrug Resistance Due to the Cell Protection by Bone Marrow Leukemia Microenvironment in Chronic Myeloid Leukemia
3747. BCR/ABL-Mediated Myeloid Expansion Is Promoted by C/EBPβ, a Regulator of Emergency Granulopoiesis
3748. Bcr-Abl Impairs T Cell Development From Murine Induced Pluripotent Stem Cells and Hematopoietic Stem Cells; A Partial Explanation for the Concept That Ph+ Clone Never Involves T Cell Lineage in CML
3749. Deregulated Activity of AML1/RUNX1 Cooperates with BCR-ABL to Immortalize Hematopoietic Progenitor Cells and Induces Blast Crisis-Like Disease of Chronic Myelogenous Leukemia in Mice
3750. The Gads Adaptor Protein Is Required to Mediate Lymphoid Disease Downstream of BCR-ABL
3751. Enrichment and Trafficking of BCR-ABL Oncoprotein in Exosomes Promotes the IL-3 Independent Proliferation of Bystander Cells
3752. Single Molecule Real Time (SMRT™) Sequencing Sensitively Detects Polyclonal and Compound BCR-ABL in Patients Who Relapse on Kinase Inhibitor Therapy
3753. Chronic Myeloid Leukemia Patients on Tyrosine Kinase Inhibitor Have Normal T Cell Responses to Vaccination but An Impaired IgM Humoral Response Associated with Loss of Discrete Memory B Cell Subsets
3754. Induced Pluripotent Stem Cells (iPSC) From Chronic Myeloid Leukemia : Study of BCR-ABL Addiction and Effect of Tyrosine Kinase Inhibitors
3755. Cytogenetic Aberration Profile of Chronic Myeloid Leukemia and Its Dynamic Changes During Imatinib Therapy
3756. A p210 BCR/ABL Mutant That Lacks Guanine Nucleotide Exchange Factor Activity Induces Erythroleukemia in a Murine Bone Marrow Transplantation Model
3757. A Novel SET Antagonist (OP449) Is Cytotoxic to CML Cells, Including the Highly-Resistant BCR-ABL^{T315I} Mutant, and Demonstrates Enhanced Efficacy in Combination with ABL Tyrosine Kinase Inhibitors
3758. Nuclear Export (Karyopherin) Inhibitors: A Novel Therapeutic Strategy for Treating Blast Crisis Chronic Myelogenous Leukemia (CML) and Philadelphia-Positive (Ph^{+}) Acute Lymphoblastic Leukemia (ALL) Through Interference with hnRNP Nucleocytoplasmic Shuttling and Rescue of Protein Phosphatase 2A (PP2A) Tumor Suppressor Activity

13 Posters: Therapy: Poster III [3759-3789]

3759. Discontinuation of Imatinib in Japanese Patients with Chronic Myeloid Leukemia
3760. Dasatinib Has a Dual Mode of Action: Direct BCR-ABL1 Mediated Anti-Leukemic Effects Are Complemented by Promotion of Th1-Type and NK-Cell Mediated Cellular Immune Responses
3761. Subcutaneous Omacetaxine in Chronic or Accelerated Chronic Myeloid Leukemia Resistant to Two or More Tyrosine-Kinase Inhibitors Including Imatinib
3762. Prediction of Molecular Response of Chronic Phase CML Patients by the EUTOS Score: Results of the Randomized CML-Study IV
3763. Upfront Imatinib in Pediatric Chronic Myeloid Leukemia Yields Results Comparable to Stem Cell Transplant
3764. Immunoprofile of Patients with Chronic Myeloid Leukemia Treated with Imatinib, Nilotinib or Dasatinib
3765. Discontinuation of Imatinib in Patients with CML and Sustained Complete Molecular Response (CMR) for Over 2 Years in the Japanese Population – An Interim Analysis of KEIO STIM Study

3766. Imatinib Long-Term Effects Study: Global Independent Assessment of Imatinib in Chronic Myeloid Leukemia: Results At 8 Years
3767. Modeling CML Development and Drug Resistance Using iPSC Technology
3768. Nilotinib Shows Safety and Efficacy in Older Patients (≥ 65 years) with Newly Diagnosed Chronic Myeloid Leukemia in Chronic Phase Comparable with That in Younger Patients with Chronic Myeloid Leukemia in Chronic Phase: Results From ENESTnd
3769. EUTOS Score Is Not Predictive for Survival and Outcome in Patients (pts) with Chronic Myeloid Leukemia in Early Chronic Phase (CML-CP) Treated with Tyrosine Kinase Inhibitors (TKIs) At MD Anderson Cancer Center (MDACC)
3770. Nilotinib in Imatinib-Resistant or -Intolerant Patients (pts) with Chronic Myeloid Leukemia in Chronic Phase (CML-CP): 48-Month Follow-up Results of a Phase 2 Study
3771. Memory Impairment in Chronic Phase (CP) Chronic Myeloid Leukemia (CML) Patients (pts) Treated with Dasatinib Tyrosine Kinase Inhibitor (TKI) Therapy
3772. High Incidence of Primary Resistance to Imatinib in the Group of Ph-Negative BCR-ABL-positive CML Patients
3773. Distinct Characteristics of e13a2 versus e14a2 BCR-ABL Chronic Myeloid Leukemia under Upfront Treatment with Imatinib – an Analysis of the German CML Study IV
3774. Analysis of the GeneXpert System on the International Multicentre ICORG 08-02 Phase II Study of Nilotinib 300mg BID as Frontline Treatment in Patients with Early Chronic Phase Chronic Myeloid Leukemia (ECPCML)
3775. Ultra-Deep Amplicon Sequencing Using Roche 454 Technology Allows High Sensitivity Bcr-Abl Kinase Domain Mutation Screening and Anticipates Emerging Mutations Leading to Resistance to Tyrosine Kinase Inhibitors in Philadelphia-Positive Leukemia Patients
3776. Cycling Toward Leukemia Stem Cell Elimination Wtih a Selective Sonic Hedgehog Antagonist
3777. Prolonged Survival Following Imatinib Failure for CML Patients in CP May Require Multiple TKI Strategies for Responding Patients
3778. Pharmacodynamic Analysis of Bosutinib in Patients with Chronic Myelogenous Leukemia
3779. Frontline Tyrosine Kinase Inhibitors (TKI) As Initial Therapy for Patients with Chronic Myeloid Leukemia in Accelerated Phase (CML-AP)
3780. Impact of Second-Generation Tyrosine Kinase Inhibitors As Second Line Treatment for Patients with Chronic Myeloid Leukemia
3781. Fluctuating Values of Molecular Residual Disease (MRD) without Molecular Progression After Imatinib Discontinuation in Patients (pts) with Chronic Myeloid Leukemia (CML) Who Have Maintained Complete Molecular Response: Implications for Re-Treatment Criteria and Role of Prior Interferon Therapy. A Pilot Study of the French CML Group (FILMC)
3782. EOSTA: An Observational Study on the Compliance and Quality of Life (QoL) of Chronic Myeloid Leukemia (CML) Patients Treated with Second Line Nilotinib (Tasigna®) : Interim Results At 6 Months of Follow-up
3783. Complete Cytogenetic Response After 3 Months Is a Very Early Indicator of Good Response to Imatinib As Front-Line Treatment in Chronic Myelogenous Leukemia
3784. Kinetics of Molecular Response with Different Tyrosine Kinase Inhibitors (TKI) Used As Frontline Therapy in Chronic Myeloid Leukemia-Chronic Phase (CML CP)
3785. Fatigue in Chronic Myelogenous Leukemia Patients (pts) Treated with Tyrosine Kinase Inhibitors (TKI)
3786. Clinical Significance of Dose Reductions of Dasatanib and Nilotinib When Used As Frontline Therapy for Chronic Phase -Chronic Myeloid Leukemia (CML-CP) Does Not Affect Outcome
3787. Outcome of Patients (pts) with Chronic Myeloid Leukemia (CML) Treated with Tyrosine Kinase Inhibitors (TKI) Who Have a History of Prior Malignancies
3788. Outcome of Complex Variant Philadelphia Positive Chromosome Translocations in the Era of Tyrosine Kinase Inhibitors
3789. The Role of Imatinib Plasma Level in the Achievment of Complete Cytogenetic Response (CCyR) in Chronic Myeloid Leukemia (CML) Therapy

14 All CML-related abstracts

http://ash.confex.com/ash/2011/webprogram/keywordindexc.html

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