



Abstracts and notes on CML presentations¹ ASH 2012 Atlanta

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1 Highlights

There were 159 abstracts about CML this year: 36 oral presentations and 123 posters. If you're pushed for time read the first couple of pages 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 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/2012/webprogram/meeting.html>

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

1. **The '10% thing' is all over the place**
2. **Getting 'even better' responses and getting off treatment**
3. **Various drugs jostling for position – no clear winner**

The '10% thing' – again...

This theme really took off this year. The story originated from Liverpool in 2003 but the large German study earlier this year from Hanfstein pushed things forward. I lost count of how many times I heard this presented at ASH. Put simply, failure to achieve a PCR level of less than 10% BCR-ABL/ABL after 3 months on a TKI (the story is expanding from just imatinib now [1678]) is associated with a less favourable outcome. Neelakantan looked at whether measuring 6 month PCR response on imatinib added anything to the <10% at 3 months story [68]. The answer seemed to be 'no', 3 months does the trick. The 10% story seems to hold true for **dasatinib** [1675] and for **nilotinib** [2797].

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There was an update on ENESTnd (**nilotinib**) [167, 1676] focusing on molecular response prediction. 33% of imatinib patients fail to get to less than 10% at 3 months whereas only 9.3% of nilotinib patients end up in the same boat. **Switching to nilotinib** from imatinib produces deeper molecular responses according to the ENESTcmr study [694] – 2 years follow up. Whether this is important in terms of survival or ability to stop remains to be seen. Peripheral arterial occlusive disease (PAOD) on **nilotinib** has been raised as a concern recently: abstracts 914 and 1679 say it's a worry, 2757 says it isn't. Hyperglycaemia is more common on nilotinib [1686] but it's not clear what the mechanism of PAOD is. Make your own mind up but it seems to me that with all the second/third gen TKIs (is ponatinib 'third' gen?) we're going to have look out for subtle but potentially important longer term side effects especially cardiovascular.

Tim Brummendorf gave an update on **bosutinib** in the BELA study, [69] again looking at the 10% at 3 months response question. Guess what, the same story comes out: getting below 10% at 3 months is important on this drug as well at least in terms of subsequent achievement of CCyR and MMR. Too early to see if survival affected.

Crucially what we don't know, with our current state of knowledge, is whether switching at 3 months if the patient is over 10% can 'rescue' them. No studies have addressed this as yet and it will be a key aspect of SPIRIT 3 in the UK.

Getting 'even better' responses, getting patients off treatment

There were a lot of presentations about this. Various strategies to get PCR down to levels where patients can stop. Is low level important with respect to outcome, particularly survival? Does it allow more patients to stop? Is there more tox to put up with if you're adding/switching drugs?

A retrospective survey from the MD Anderson [164] of patients treated with **imatinib, nilotinib and dasatinib** shed some light on the question of whether achievement of MR3 (MMR) predicts for survival. Although the differences are not large there's a statistically significant benefit on OS in achieving MMR within 18-24 months. These data are retrospective and don't of course address the question of whether switching drugs to obtain MR3 has a similar effect. Studies need to be done to investigate this further.

The Adelaide group added to this [165]: they suggest in **imatinib**-treated patients that 3 month PCR level, speed of response and gender are important. Women do better: maybe they're better at taking the pills? OCT-1 activity may also be informative [693]. These factors might also contribute to our understanding of who might be able to stop treatment. Data along similar lines (although less mature) were presented from the ENESTnd study [167]. Whether the MMR 'hares or tortoises' differ in survival terms remains to be seen.

The German CML group have found that **dose-optimised** (between 400-800mg) **imatinib** produces more CMR^{4,5} responses and a landmark analysis at 48 month indicated better overall survival in these patients and of course these patients might be able to stop treatment [67].

Cytogenetics may still have a role to play in predicting outcome and a large study (1346 patients) from Germany [913] looked at this. Patients with major route additional chromosomal alterations (major ACA: +8, i(17)(q10), +19, +der(22)t(9;22)(q34;q11) have a worse outcome whereas patients with minor route ACA show no difference in overall survival (OS) and progression-free survival (PFS) compared to patients with the standard translocation, a variant translocation or the loss of the Y chromosome.

Next gen sequencing is coming to the fore in CML as everywhere else. The Italian group have used '**ultra-deep**' sequencing to look at TK domain mutations as potential predictor of response to different drugs [692]. The answer? It seems to get more complicated (confusing?) with 'better' technology and it's not clear to me that mutation analysis (perhaps apart from T315I) is that compelling when it comes to choosing TKI drugs. SMRT sequencing has also been used [917].

Lots of abstracts on **stopping imatinib** [e.g. 2788, 1684 – with maintenance IFN, 4274] including one looking at patient preferences [4274]. Nobody has yet explored dose reduction rather than stopping or the possibility of reducing dose from MR3: we're planning to look at this in the DESTINY study in the UK. Delphine Rea updated the French experience of **stopping 2nd generation TKIs** [916]. This study, although still early, seem to confirm what you might expect: higher rates of sustained negativity than with imatinib in the 39 patients with a minimum of 6 months follow up. 60% in the study were female and it was about 50:50 dasatinib:nilotinib although only 2 patients received these as first line therapy. 16 of 39 (41%) lost MMR (loss of MMR rather than CMR was used) most by 8 months but one at 24 months. All relapsers were fine when treatment restarted. Freedom from relapse was much better in those who were on 2nd gen first line or for imatinib intolerance rather than imatinib resistance: 68% vs 40% relapse free at 1 year. So, my simple take on the story so far: previous STIM study

(imatinib) ~40% can stay off treatment; on 2nd gen about 60% can stay off. Will be interesting to watch this story develop: will 2G drugs prove their worth in allowing more CML patients to stop treatment?

Jostling for position

There were updates on the primary clinical outcomes from some of the main second generation studies DASISION [1675, **dasatinib**], ENESTnd [1676, **nilotinib**], BELA [69, **bosutinib**] although emphasis was mainly on early response – I suspect we'll get more comprehensive updates at ASCO in June 2013. I don't think the story has changed since last year: undoubtedly better PCR responses but no difference in overall survival. I'm increasingly thinking there might be a trade off with the newer drugs: you will achieve a better response but this might be at the risk of other side effects, especially cardiovascular. We need to keep weighing these factors in the balance.

There was an update on the PACE study (**ponatinib**) with 12 months follow up [163]. This study enrolled 449 patients who had failed nilotinib or dasatinib; 234 were in chronic phase without T315I, 63 had T315I. CCR rate was 46% overall, MMR was 34% (91% remained in MMR). PFS at 1 year is 80%, OS is 94%. Pretty good in quite a difficult group of patients. Common side effects appeared to be rash, thrombocytopenia. Less commonly pancreatitis and hypertension. Phase 1 study (pre-PACE) was in NEJM on 29th Nov. Abstract [3763] described PCR response data and advanced phase use was also presented [915].

Unlike in the UK, our French colleagues continue to be interested in **interferon alpha** and as well as an update of the French SPIRIT study [168] data from an early phase nilotinib plus IFN study were presented [166]. A phase 3 trial (nilotinib +/- IFN) might follow. The French SPIRIT study has offered an interesting insight: the '10% at 3 months' landmark that has received so much attention in the last year or two seems less relevant in predicting progression in patients treated with higher-dose imatinib or imatinib with interferon. Effects, if any, on overall survival are not known as yet – it's too early. There was a 30 year follow up from the MD Anderson on patients treated with IFN [918].

A Japanese group have confirmed the observation that early lymphocytosis on **dasatinib** reliably predicts for subsequent complete molecular response – mechanism not known [691].

Radotinib seems to have come out of the blue. Also called 'Supect' or IY5511 from Il-Yang Pharma in Korea [695] the dosing looks similar to nilotinib. There's an ongoing phase 3 trial comparing with imatinib. Interesting to see whether this one takes off elsewhere in the world. Could it compete on price?

An interesting study from France piloted the use of the PPAR- γ agonist **pioglitazone** (the diabetes drug) which can reduce activity of *Stat5A/B*. 27 patients with very good molecular response were given this drug in addition to imatinib to see if they could be rendered PCR-negative [696]. Early days but 3 patients so far have become PCR negative. Trouble is that pioglitazone appears to be associated with the development of bladder cancer.

As **imatinib** goes off patent in 2016 in the UK (earlier in some countries e.g. Canada, New Zealand) it's interesting to watch the development of (lots of) generics. So far there's at least ten: Genfatinib, Imatinib Teva, Veenat, Celonib, Imatib, Mesylonib, Mitinab, Shantinib, Zoleta, Spotnib. What will happen in 2016 is anyone's guess: there have been no set patterns of price reduction when other drugs have gone off patent but sometimes price has dropped by 90%.

A few other things caught my eye

KPT-330 looks interesting. A 'CRM1-inhibitor', it's the first selective inhibitor of nuclear export (SINE) and the group from Columbus, Ohio presented some initial *in vitro* data showing that it inhibits CML cells [35]. Different mechanism of action than TKIs and not yet in clinical trials. **Targetting RAD52** might be a useful to eliminate CML stem cells [3, plenary session]. Lots more than I can summarise here of course – have a browse through the abstracts in the rest of the report. Hope you found my ramblings useful.

2 Plenary session [3]

[3] RAD52-Dependent Synthetic Lethality Eradicates Leukemia Stem Cells. Cramer. We showed that imatinib-naive and imatinib-treated BCR-ABL1 kinase-positive chronic myeloid leukemia in chronic phase (CML-CP) Lin⁻CD34⁺CD38⁻ stem cells (LSCs) and Lin⁻CD34⁺CD38⁺ leukemia progenitor cells (LPCs) accumulate high numbers of the reactive oxygen species (ROS)-induced DNA double-strand breaks (DSBs) which, if not repaired, are lethal (Nieborowska-Skorska et al., Blood, 2012). Genome-wide microarray screen indicated that LSCs overexpress numerous genes responsible for homologous recombination repair (HRR) of DSBs. Direct targeting of RAD51, a key element in HRR, by small molecule inhibitor exerted anti-CML effect, but normal cells were also affected, indicating that RAD51 is not a preferable target. In normal cells HRR depends primarily on BRCA1/BRCA2-RAD51 pathway, while RAD52-RAD51 pathway may serve as a back-up. However BRCA1 protein is downregulated in CML, and RAD51(F259V) mutant (RAD52 binding-deficient) induced apoptosis and reduced cell growth in BCR-ABL1 –dependent manner, thus highlighting the potential role of alternative RAD52-driven HRR in CML. The absence of RAD52 (*Rad52*^{-/-}) inhibited BCR-ABL1 –mediated cell cycle progression and clonogenic activity, induced apoptosis, reduced Lin⁻Kit⁺Sca1⁺CD34⁺Fit3⁻ long-term LSCs and Lin⁻Kit⁺Sca1⁺CD34⁺Fit3⁻ short-term LSCs, and abrogated leukemogenesis in murine model of CML. At the same time RAD52 was expendable in normal cells. In addition, RAD52 was essential to prevent accumulation of ROS-induced lethal DSBs in BCR-ABL1 –positive murine LSCs. Thus it appears that RAD52 is necessary to repair numerous ROS-induced DSBs in LSCs to promote leukemogenesis. BCR-ABL1 kinase interacts with and phosphorylates RAD52 on Y104, but phosphorylation-deficient RAD52(Y104F) mutant did not exert any negative effect on BCR-ABL1 kinase-driven leukemogenesis. This observation suggests that RAD52 activity is preserved in imatinib-treated cells. On the other hand, DNA binding-deficient RAD52(F79A) and RAD52(K102A) mutants (disrupt DNA binding domain I and II, respectively) inhibited clonogenic potential of Lin⁻CD34⁺ CML-CP cells and BCR-ABL1 –positive Lin⁻Kit⁺Sca1⁺ murine LSCs, but not normal counterparts. Therefore, RAD52-mediated DNA binding activity appears to be essential for BCR-ABL1 kinase-driven leukemogenesis, but not normal hematopoiesis. RAD52 DNA I binding groove containing F79 forms a pocket/niche. Peptide aptamer containing amino acid residues surrounding RAD52 F79, but not that with F79A substitution, inhibited RAD52 foci, RAD52-dependent RAD51 foci and HRR activity, resulting in elevation of the number of lethal DSBs and abrogation of clonogenic activity of BCR-ABL1-positive murine leukemia cells. Aptamer F79 reduced the growth of LSCs and LPCs from CML-CP and more aggressive CML-accelerated phase (CML-AP), depleted quiescent LSCs by attrition and eradicated BCR-ABL1 leukemia from SCID mice. Moreover, aptamer F79 enhanced the anti-CML effects of imatinib. The effect of aptamer F79 in BCR-ABL1 leukemia cells depended on downregulation of BRCA1 implicating “synthetic lethality”. At the same time the aptamer did not exert any measurable negative effects on normal cells and tissues. In conclusion, we postulate that CML-CP/AP cells are “addicted” to RAD52 and that targeting of DNA binding domain of RAD52 may induce “synthetic lethality” to eliminate proliferating LSCs/LPCs and to deplete quiescent LSCs. Moreover, anti-RAD52 F79 aptamer exerted anti-tumor activity in acute myeloid leukemia primary cells, and in cell lines derived from carcinomas of breast, ovary and pancreas displaying BRCA1 and/or BRCA2 deficiency. Thus, BRCA1/BRCA2ness-driven “addiction” to RAD52 may be selectively targeted to achieve “synthetic lethality” in wide-range of tumors while being harmless to normal cells.

3 CML Therapy I [163-168]

[163] A Pivotal Phase 2 Trial of Ponatinib in Patients with Chronic Myeloid Leukemia (CML) and Philadelphia Chromosome-Positive Acute Lymphoblastic Leukemia (Ph+ALL) Resistant or Intolerant to Dasatinib or Nilotinib, or with the T315I BCR-ABL Mutation: 12-Month Follow-up of the PACE Trial. Cortes, Houston. Background: Despite progress in Ph+ leukemia therapy, patients who experience failure of tyrosine kinase inhibitors (TKIs) and those with the T315I BCR-ABL mutation have limited treatment options. Ponatinib is an oral TKI developed using computational and structure-based design with optimal binding to the BCR-ABL active site. At clinically achievable concentrations, ponatinib demonstrated potent in vitro activity against native BCR-ABL and all BCR-ABL mutants tested, including T315I. The efficacy and safety of ponatinib (45 mg orally once daily) in patients with Ph+ leukemia were evaluated in a phase 2, international, open-label clinical trial. **Methods:** 449 patients resistant or intolerant (R/I) to dasatinib or nilotinib or with the T315I mutation confirmed at entry were enrolled and assigned to 1 of 6 cohorts: chronic phase (CP)-CML R/I (N=203), CP-CML T315I (N=64), accelerated phase (AP)-CML R/I (N=65), AP-CML T315I (N=18), blast phase (BP)-CML/Ph+ALL R/I (N=48), BP-CML/Ph+ALL T315I (N=46). Five patients (3 CP-CML, 2 AP-CML) without confirmed T315I and not R/I to dasatinib or nilotinib were treated, but not assigned to a cohort; they were included in safety analyses. The primary endpoint was major cytogenetic response (MCyR) at any time within 12 months for CP-CML and major hematologic response (MaHR) at any time within 6 months for advanced Ph+ leukemia. The trial is ongoing. Data as of 23 July 2012 are reported: median follow-up 11 (0.1 to 21) months; minimum follow-up 9 months. **Results:** Median age was 59 (18-94) yrs; 53% were male. Median time from diagnosis to ponatinib was 6 (0.3-28) yrs. Patients were heavily pretreated: 96% received prior imatinib, 84% dasatinib, 65% nilotinib; median number of prior TKIs was 3, with 53% exposed to all 3 approved TKIs. In patients previously treated with dasatinib or nilotinib (N=427), 88% had a history of resistance and 12% were purely intolerant to dasatinib or nilotinib. Best prior response to most recent dasatinib or nilotinib was 26% MCyR or better in CP-CML, and 23% MaHR or better in advanced Ph+ leukemia. Frequent BCR-ABL mutations confirmed at entry were: 29% T315I, 8% F317L, 4% E255K, 4% F359V, 3% G250E. No mutations were detected in 44%. The primary endpoint response rates (see Table) in each cohort exceeded the prespecified statistical criteria for success. In CP-CML and AP-CML R/I (the 3 largest cohorts), 95% CIs exceeded the prespecified response rate. Median time to response (for responders) was 84 days in CP-CML, 112 days in AP-CML, 55 days in BP-CML/Ph+ALL. Responses were durable; the estimated (Kaplan-Meier) probability of responders maintaining the primary endpoint at 1 yr was 91% in CP-CML, 42% in AP-CML, 35% in BP-CML/Ph+ALL. In CP-CML, 46% had complete cytogenetic response and molecular response rates were 32% MMR, 20% MR4, and 12%

MR4.5. Response rates were higher in patients exposed to fewer prior TKIs and those with shorter disease duration. Similar response rates were observed in patients with and without BCR-ABL mutations. In CP-CML, response rates were higher in those with T315I; however, a post hoc analysis found that presence of T315I was not a predictor of response. Instead, the difference in response rate was explained by T315I patients' younger age, shorter duration of leukemia, and exposure to less prior therapy. At the time of analysis, 52% of patients remained on therapy (66% CP-CML). The most frequent reasons for discontinuation were progression (18%) and AEs (12%). The most common drug-related AEs were thrombocytopenia (36%), rash (33%), and dry skin (31%). Pancreatitis was the most common drug-related SAE (5%); however, it occurred early and was managed with dose modification (1 patient discontinued due to pancreatitis). **Conclusions:** Ponatinib has substantial activity and is generally well tolerated in these heavily pretreated Ph+ leukemia patients who have limited available treatment options. Data with a minimum follow-up of 12 months will be presented. **NOTES:** 23% any and 7% grade 3/4 hypertension.

Primary Endpoint	CP-CML		AP-CML		BP-CML/Ph+ALL	
	MCyR		MaHR		MaHR	
	Response n/N (%)	95% CI	Response n/N (%)	95% CI	Response n/N (%)	95% CI
R/I	101/203 (50)	43-57	38/65 (58)	46-71	17/48 (35)	22-51
T315I	45/64 (70)	58-81	9/18 (50)	26-74	15/46 (33)	20-48
Total	146/267 (55)	49-61	47/83 (57)	45-68	32/94 (34)	25-45

Baseline MaHR counted as non-responder. Prespecified null and interesting rates: CP-CML R/I, MCyR 20% and 35%; CP-CML T315I, MCyR 10% and 35%; advanced cohorts, MaHR 10% and 30%.

[164] Clinical Significance of Deeper Molecular Responses with Four Modalities of Tyrosine Kinase Inhibitors As Frontline Therapy for Chronic Myeloid Leukemia. Falchi, Houston. Background: The achievement of a major molecular remission (MMR) after imatinib therapy in pts with chronic myeloid leukemia (CML) in chronic phase (CP) predicts for decreased risk of events, but has little impact in overall survival (OS) among patients with complete cytogenetic response (CCyR). Deeper molecular responses (MR), including undetectable transcripts, are frequently sought in patients with CML treated with tyrosine kinase inhibitors (TKI), but the prognostic significance of these responses is not known. **Objectives:** To determine the long-term clinical significance of achieving deeper level of MR achieved after therapy with TKI for CML in CP. **Methods:** Pts were included in clinical trials for initial therapy for CML with one of the following modalities: imatinib 400mg/day (IM400), imatinib 800mg/day (IM800), nilotinib (NILO) and dasatinib (DASA). We defined the level of MR as MMR, MR4, MR4.5 and undetectable transcripts (UND), corresponding to an ABL/BCR-ABL ratio (International Scale) of $\leq 0.1\%$, $\leq 0.01\%$, $\leq 0.0032\%$, and undetectable transcripts (minimum sensitivity 4.5-log), respectively. **Results:** A total of 495 pts were treated: 83 pts with IM400, 204 with IM800, 106 with NILO and 102 with DASA. At presentation leukocyte counts were higher in the NILO group (41.5 vs 22.2, 27.5 and $27 \times 10^9/L$ for IM400, IM800 and DASA pts). All other patient characteristics were equally distributed across the 4 treatment groups. After a median follow-up of 73 months (2 to 142), complete cytogenetic response (CCyR) was achieved in 88%. CCyR rates for IM400, IM800, NILO and DASA pts were 82%, 88%, 90% and 90%, respectively. Best level of MR for the entire population was: <MMR in 17% of pts, MMR in 13%, MR4 in 5%, MR4.5 in 19%, UND in 44%. In IM400 pts MR was <MMR in 28% of pts, MMR in 10%, MR4 in 8%, MR4.5 in 14%, UND in 40%. In IM800 pts MR was <MMR in 14% of pts, MMR in 8%, MR4 in 5%, MR4.5 in 19%, UND in 54%. In NILO pts MR was <MMR in 18% of pts, MMR in 20%, MR4 in 7%, MR4.5 in 22%, UND in 33%. In DASA pts MR was <MMR in 18% of pts, MMR in 18%, MR4 in 7%, MR4.5 in 23%, UND in 39%. There was a trend for earlier achievement of MR with NILO: median times to MMR, MR4, MR4.5 and UND were 12, 17.4, 17.9 and 25.1 months, respectively, for IM400 pts; 5.8, 8.7, 11.8 and 23.7 months, respectively, for IM800 pts; 5.7, 7, 8.3 and 16.4 months, respectively, for NILO pts; 5.7, 8.8, 17.4 and 27.2 months, respectively, for DASA pts. To analyze the relationship between the degree of MR and clinical outcome we excluded pts not achieving a CCyR as their best response since the clinical significance of CCyR is well known. For the remaining 438 pts, the depth of molecular remission was inversely correlated with the risk of losing CCyR (19%, 16%, 11%, 7%, 2% in pts with <MMR, MMR, MR4, MR4.5 and UND, respectively) or losing MMR (31%, 42%, 24%, 2%, respectively), as well as the risk of events (22%, 20%, 15%, 12%, 3%, respectively), transformation (3%, 5%, 0%, 1%, 0%, respectively), or death (25%, 11%, 8%, 6%, 4%, respectively). The 6-year OS for pts with <MMR, MMR, MR4, MR4.5 and UND is 74%, 84%, 95%, 96% and 99%, respectively ($p < .0001$); transformation-free survival (TFS) is 95%, 93%, 100%, 100% and 100%, respectively ($p < .014$); event-free survival (EFS) is 74%, 74%, 86%, 89% and 99%, respectively ($p < .0001$). To adjust for the lead-time to achieve deeper responses, we then calculated OS, TFS and EFS rates at 6 years according to the depth of molecular response at 18 or 24 months. Results are summarized in table 1. **Conclusion:** Most patients treated with TKI as initial therapy for early CP CML achieve a MR during the course of treatment. BCR-ABL transcripts become undetectable in a significant fraction of them. Achieving a MMR or better at 18 months or 24 months is associated with significantly superior 6-years OS, TFS and EFS. These result suggest that deeper molecular responses (MMR and beyond) are associated with clinical benefit, with a particularly good outcome for those achieving undetectable transcript levels.

Table 1. Survival at 6 years by level of molecular response at 18- and 24-month landmarks.

Outcome	Molecular response at 18 months					p-value
	No MR	MMR	MR4	MR4.5	UND	
6-years OS	93%	98%	97%	95%	100%	.153
6-years TFS	90%	100%	100%	100%	100%	.03
6-years EFS	78%	94%	97%	93%	100%	<.0001
Outcome	Molecular response at 24 months					p-value
	No MR	MMR	MR4	MR4.5	UND	
6-years OS	92%	97%	100%	97%	100%	.018

6-years TFS	90%	100%	100%	100%	100%	.057
6-years EFS	80%	90%	97%	95%	100%	<.0001

[165] Early Molecular Response and Female Sex Strongly Predict Achievement of Stable Undetectable *BCR-ABL1*, a Criterion for Imatinib Discontinuation in Patients with CML. Branford, Adelaide. Introduction. The opportunity to discontinue kinase inhibitor therapy while maintaining a deep remission is desirable for many CML patients. Despite good responses to imatinib for most patients, treatment related side effects remain problematic and can affect quality of life. Studies have demonstrated that a proportion of carefully selected patients can sustain response after imatinib discontinuation. The first requirement for successful discontinuation is likely to be stable deep molecular response based on a sensitive RQ-PCR assay. The criteria for patient selection in the French Stop Imatinib (STIM) and Australian CML8 (TWISTER) imatinib discontinuation trials included stable undetectable *BCR-ABL1* transcripts for at least 24 months with a PCR sensitivity of 5 and 4.5 log, respectively. The probability of continued remission after discontinuation for imatinib treated patients without prior interferon- α therapy was approximately 33%. It is not known how many imatinib treated patients will eventually meet these PCR criteria for a discontinuation trial. **Aims.** We aimed to determine 1) the cumulative probability of achieving the PCR criteria for imatinib discontinuation as defined in the CML8 study, and 2) factors that predicted its achievement. **Method.** The molecular response of 415 de-novo CML patients in chronic phase enrolled in consecutive clinical trials of imatinib since July 2000 was examined. The assigned daily imatinib dose was 400 mg for 90 patients, 600 mg for 202 patients and 800 mg for 123 patients. Molecular data were included until imatinib cessation or last follow-up. The minimum time since commencing imatinib was 30 months and the median time on imatinib was 45 months, range 3 to 136. The CML8 PCR criteria for imatinib discontinuation were confirmed undetectable *BCR-ABL1* transcripts at a sensitivity of 4.5 log that remained undetectable on all PCR tests for at least 24 months while on imatinib therapy. In the current analysis the CML8 PCR discontinuation criteria are defined as 'stable UMR^{4.5}'. **Results.** At 8 years of imatinib therapy the cumulative incidence of stable UMR^{4.5} was 43%, Figure A. Patients were divided into groups according to the time to a confirmed major molecular response (MMR): by 3, 6, 12 or 18 months. There was a significant difference in stable UMR^{4.5}, $P < .001$, Figure B. The cumulative incidence of stable UMR^{4.5} was more than 60% for all patients who achieved MMR by 12 months and only 16% for patients with MMR between 12 and 18 months. The time to a confirmed MMR influenced the time to reach a stable UMR^{4.5} after achieving MMR. Considering only patients who achieved stable UMR^{4.5}, patients achieving MMR by 3 months took a further 39 months (median) to achieve stable UMR^{4.5}. For those with MMR by 6 months and 12 months, the median month to a stable UMR^{4.5} was 50 and 76 months after MMR, $P < .001$. This suggests slower dynamics of *BCR-ABL1* decline with delayed time to MMR. 52 patients achieved MMR after 18 months and none achieved a stable UMR^{4.5} by 8 years: median time to MMR was 27 months, range 21-87. Factors at the time of commencing imatinib (baseline) were examined for their association with stable UMR^{4.5}; Sokal risk, age, sex, assigned imatinib dose and baseline *BCR-ABL1* value, as well as the 3 month *BCR-ABL1* value. Quantitative factors were categorized into groups, with cut-offs set at the median for age and quartiles for the baseline *BCR-ABL1* value. By univariate analysis the only baseline factor that predicted for higher cumulative incidence of stable UMR^{4.5} at 8 years was female versus male, 68% versus 30%, $P < .001$, Figure C. During imatinib therapy females had significantly lower median *BCR-ABL1* values at every assessment up to 42 months. The 3 month *BCR-ABL1* value also predicted stable UMR^{4.5}, $P < .001$, Figure D. Baseline and 3 month factors were entered into a multivariate analysis. The 3 month *BCR-ABL1* value and sex were independent predictors of stable UMR^{4.5}, $P = .004$ and $P = .005$, respectively. **Conclusion.** The time to achieve an MMR, sex and the 3 month *BCR-ABL1* value predicted stable undetectable *BCR-ABL1* while on imatinib. Lower *BCR-ABL1* values and higher rates of stable UMR^{4.5} in females could be related to better drug adherence or biological differences. Further studies are indicated. Early MMR led to early achievement of the discontinuation criteria. The findings justify the focus on early achievement of MMR as a strategy to maximize recruitment to discontinuation studies.

[166] Pegylated Interferon- α 2a in Combination to Nilotinib As First Line Therapy in Newly Diagnosed Chronic Phase Chronic Myelogenous Leukemia Provides High Rates of MR^{4.5}. Preliminary Results of a Phase II Study. Nicolini, Lyon. Background. Imatinib mesylate combined to pegylated interferon alfa 2a (Peg-IFN) has been reported to significantly enhance the molecular responses for *de novo* chronic phase chronic myeloid leukemia (CP-CML) patients compared to Imatinib alone in a Phase 3 study (Preudhomme et al. NEJM 2010). Second generation tyrosine kinase inhibitors (TKI2) such as nilotinib induce significantly higher levels of cytogenetic and molecular responses than imatinib as front line therapy for CP-CML (Saglio et al., NEJM 2010). **Aims.** Test the combination of nilotinib + Peg-IFN as front line therapy in CP-CML patients in order to check the safety and evaluate the molecular response rates (EudraCT 2010-019786-28). **Methods.** In this 2-step French national study, patients were assigned first to Peg-IFN (\pm HU) for a month at 90 mg/wk prior to a combination of nilotinib 300 mg BID + Peg-IFN 45 mg/wk for ≥ 1 year. The primary endpoint was the rate of confirmed (on 2 datapoints) molecular response 4.5 (MR^{4.5}) by 1 year. Molecular assessments were centralised for all patients and expressed as *BCR-ABL*^{IS} in %. **Results.** In the first cohort, 40+1 patients (1 screen failure) were enrolled and a second cohort of 20 patients was planned once the last patient of cohort 1 attained 1 year of treatment, if the primary endpoint would have not been reached. The current median follow-up is 13.6 (10.1–16.3) months. Sokal and Euro scores were high for 12% and 2%, intermediate for 49% and 55% and low for 39% and 43% of the patients respectively. Euro score was high for one patient. The median age was 53 (23-85) years. Two patients had a masked Philadelphia chromosome, 3 a variant form, and 1 had additional chromosomal abnormalities, all patients had a "major" BCR transcript. Five percent of patients were in CHR at 1 month of Peg-IFN and 100% at month (M) 2 (after 1 month of combination therapy). The rates of Complete Cytogenetic Responses (CCyR) at 3, 6, and 12 months of combination (i. e. at 2, 5, 8 and 11 months of TKI2) were 47%, 71%, 100% respectively on evaluable samples. The incidence of molecular responses are mentioned in figure 1 Figure 1: Incidence of molecular responses at definite time points.

Of note, 87% of the patients had a *BCR-ABL*^{IS} $\leq 10\%$ at M3. The rates of molecular responses broke down by major molecular response (MMR): 27%, 4 log reduction (MR⁴): 36%, and ≥ 4.5 log *BCR-ABL* reduction (MR^{4.5}, MR⁵ and undetectable): 21% with a total number of 84% patients in \geq MMR and beyond (17.5% and 67.5% in intention-to-treat respectively) at 1 year. Confirmed molecular results at 1 year will be presented. Nilotinib trough levels centrally analysed at

M3, 6 and 12 for the vast majority of patients were ≥ 1000 ng/ml and Peg-IFN did not seem to impact on its pharmacokinetics. One patient went on unmutated myeloid blast crisis at M6 and is alive after allogeneic stem cell transplantation. Four additional patients were withdrawn from study: At M2 for non observance, at M6 for seizures related to an extra-dural hematoma, at M6 for recurrent grade 3 hepatic toxicity, at M9 for recurrent grade 3 pruritus. The median dose of Peg-IFN delivered to the patients during the first month was 90 (0-180) mg/wk, 45 mg/wk at M2, 3, 9, 12, and 33.75 mg/wk at M6. The median doses of nilotinib delivered to the patients were 600 mg daily at M2, 3, 6, 9, 12 and 15 as initially planned. The rate of grade 3-4 hematologic toxicities overall were anemia 2.5%, thrombocytopenia 41%, neutropenia 41% and pancytopenia 5%. These were observed mainly during M2 (16% neutropenia, 24% thrombocytopenia, 3% anemia), M3 (16% neutropenia, 13% thrombocytopenia, 3% pancytopenia) and M6 (12.5% neutropenia, 5% thrombocytopenia) and disappeared thereafter. Grade 3-4 toxicities occurred mostly during the first 3 months with 15% cholestatic episodes, 5% of ALAT elevation, 2.5% of lipase elevation, 2.5% arthro-myalgias, 2.5% abdominal pain without lipase elevation, 2.5% of depression. No PAO was observed and, to date, no dyslipidemia. **Conclusion.** The combination of nilotinib and Peg-IFN seems relatively well tolerated despite frequent initial and transient hematologic and hepatic toxicities, and provides very high rates of molecular responses at 1 year and beyond. According to the initial methodology of this trial, the second cohort of patients will not be enrolled as the MR^{4.5} rates at M12 are beyond the initial expectations. A randomised phase III study testing nilotinib versus nilotinib + Peg-IFN is warranted.

[167] Outcome of Patients with Chronic Myeloid Leukemia in Chronic Phase (CML-CP) Based On Early Molecular Response and Factors Associated with Early Response: 4-Year Follow-up Data From Enestnd (Evaluating Nilotinib Efficacy and Safety in Clinical Trials Newly Diagnosed Patients). Hochhaus, Jena. Background: In the ENESTnd study, nilotinib significantly reduced progression to accelerated phase/blast crisis (AP/BC) and demonstrated superior rates of deep molecular response vs imatinib. Data from ENESTnd demonstrated that significantly more patients achieved early molecular response of both $< 10\%$ and $< 1\%$ BCR-ABL^{IS} at both 3 and 6 months on nilotinib vs imatinib. Here, we report landmark analyses based on BCR-ABL transcript levels at 3 and 6 months using data with a minimum follow-up of 3 years and also provide data on factors associated with poor early molecular response; data based on longer follow-up of 4 years will be presented. **Methods:** The nilotinib 300 mg twice daily (BID; n = 282) and imatinib 400 mg once daily (QD; n = 283) arms from ENESTnd were used for this analysis. Patients were grouped based on BCR-ABL transcript levels of $\leq 1\%$, $> 1\%$ to $\leq 10\%$, and $> 10\%$ at 3 months (n = 258 and n = 264 patients with available PCR samples at 3 months in the nilotinib and imatinib arms, respectively) and at 6 months (n = 257 and n = 256 patients with available PCR samples at 6 months in the nilotinib and imatinib arms, respectively). Rates of major molecular response (MMR; $\leq 0.1\%$ BCR-ABL^{IS}) and molecular response with a 4.5-log reduction in BCR-ABL transcript levels (MR^{4.5}, $\leq 0.0032\%$ ^{IS}) as well as rates of progression-free survival (PFS) and overall survival (OS) were evaluated among patients grouped according to their BCR-ABL transcript levels at 3 and 6 months. Data on selected baseline characteristics and dose intensity were also assessed. **Results:** Among evaluable patients at 3 months, 9% of patients (n = 24) in the nilotinib arm vs 33% (n = 88) in the imatinib arm had BCR-ABL transcript levels of $> 10\%$; among evaluable patients at 6 months, 3% of patients (n = 7) in the nilotinib arm vs 16% (n = 40) in the imatinib arm had BCR-ABL transcript levels of $> 10\%$. Patients with a BCR-ABL transcript level of $> 10\%$ had a lower probability of future MMR or MR^{4.5} as well as poorer PFS and OS compared with patients who had BCR-ABL transcript levels $\leq 10\%$ at 3 months (Table). Results were similar based on 6-month landmark analyses. In patients with $> 10\%$ BCR-ABL transcript levels at 3 months, the average dose intensity of nilotinib within the first 3 months was 474 mg/day compared with 600 mg/day for patients with $\leq 10\%$ BCR-ABL transcript levels; the average dose intensity of imatinib within the first 3 months was the same (400 mg/day) for patients with both $\leq 10\%$ and $> 10\%$ BCR-ABL levels at 3 months (Table). Patients with $> 10\%$ BCR-ABL transcript levels at 3 months were also more likely to have high Sokal risk, larger spleen size, and additional chromosomal abnormalities compared with patients with $\leq 10\%$ BCR-ABL transcript levels at 3 months. Other factors associated with early response and further data on long-term outcomes are being assessed and will be presented with a minimum follow-up of 4 years. **Conclusions:** Fewer patients in the nilotinib arm vs the imatinib arm had BCR-ABL transcript levels $> 10\%$ at 3 and 6 months. Reasons for poor early response appeared to be related, at least in part, to baseline factors and dose intensity. Early molecular response at 3 and 6 months correlated with future MMR and MR^{4.5} as well as an increased probability of PFS and OS. Nilotinib frontline therapy allows more patients to achieve deeper responses earlier, associated with improved long-term outcomes vs imatinib.

	Nilotinib 300 mg BID (N = 258)*		Imatinib 400 mg QD (N = 264)*	
BCR-ABL at 3 months	$\leq 10\%$ n = 234	$> 10\%$ n = 24	$\leq 10\%$ n = 176	$> 10\%$ n = 88
MMR	n = 209	n = 24	n = 174	n = 88
by 1 year, %	61	4	40	2
by 2 years, %	80	29	58	21
MR ^{4.5}	n = 233	n = 24	n = 176	n = 88
by 2 years, %	29	4	14	0
by 3 years, %	38	4	24	1
Long-term outcomes	n = 234	n = 24	n = 176	n = 88
PFS at 3 years, %	95.9	82.9	97.7	83.8
OS at 3 years, %	97.6	86.7	98.9	84.8
Factors associated with molecular response at 3 months				
Median dose intensity within the first 3 months, mg/day	600	474	400	400
High Sokal risk, %	25.6	41.7	17.6	44.3
Splenomegaly, %	38.9	75.0	26.7	59.1
Spleen size, cm (median)	5.0	8.0	3.5	10.5

Additional chromosomal abnormalities, %	9.4	16.7	9.1	14.8
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Response and outcomes were evaluated in patients who had available PCR samples at 3 or 6 months. Patients who achieved the target response within 3 or 6 months were excluded from the respective analysis of response outcomes; patients who had events or were censored within 3 or 6 months were excluded from the respective analysis of time-to-event outcomes.

[168] Relationship Between Molecular Responses and Disease Progression in Patients (Pts) Treated First Line with Imatinib (Im) Based Regimens: Impact of Treatment Arm within the French Spirit Trial From the French CML Group (FI LMC). Rousselot, Versailles. The SPIRIT phase III randomized multicenter open-label prospective trial was designed to compare 4-arms, IM-400 mg versus IM-600mg versus IM-400 mg + cytarabine at a dose of 20 mg / m² / day in cycles of 28 days, versus IM-400 mg + PegIFN at an initial dose of 90 µg per week. The planned molecular analysis after 1 year based on the outcome of 636 pts resulted in a highly significant superiority of superior molecular response (SMR) (0.01 % Bcr-Abl/Abl on IS) of the combination IM 400mg-PegIFN (N Engl J Med, 2010). As of December 31st 2010, date for closing accrual, 787 pts have been included. The current analysis provides update of the trial and additional information on relationship between molecular response and outcomes. **Methods:** Progression free survival (PFS) was defined by absence of accelerated phase (AP), blast crisis (BC), and death from any reasons, whichever came first. Rates were estimated by the Kaplan-Meier method and compared within groups by the log-rank test. In addition, time to progression (TTP) being defined as AP-BC only, was estimated by cumulative incidence function and compared within groups by the Gray test. Deaths unrelated with progression were then considered as competing events. Pts with available PCR samples at 3 months (N= 665 overall, 197 IM-400mg, 147 IM-600mg, 138 IM-AraC, 183 IM-PegIFN) were classified according to the BCR-ABL cut-off level of 10% IS at 3 and 6 months. Analyses of long term outcomes were then based on the kinetic of molecular response. **Results.** After a median follow-up of 68 months, out of the 787 pts, 59 PFS events (31 AP-BC; 28 deaths in CP) were recorded. Survival without progression at 60 months were for the IM-400mg, IM-600mg, IM-400mg + cytarabine and IM-400mg + PegIFN , 94% , 93%, 90% and 94% respectively (overall, p= 0.24). However we noticed that kinetic of molecular responses of pts who experienced AP-BC was very heterogeneous as showed in Fig 1. The accurate level of bcr-abl/abl transcript and the relevance of the IS conversion factor are questionable when values are above 10% or very low. Thus corresponding plots are shadowed in the Fig1. Then, when pts were stratified according to their molecular response at 3 months, 14 cases of AP-BC and 13 deaths without evidence of progression were recorded in the group of pts with a BCR-ABL ratio <=10% IS (n=522) whereas 14 cases of AP-BC and 9 deaths were recorded in the other group (n=143). Overall, PFS at 3 months was significantly better (p <0.0001) in pts with ratio ≤10% IS. Similarly, TTP was lower (p <0.0001). However, when same analyses were performed according to treatment arm, discrepancies were observed. The potential interest of the 10% BCR-ABL cut-off was still relevant in the IM-400 arm (p <0.0001) and IM-AraC arm (p=0.0199), but no statistical differences were observed in the IM-600 (p=0.6715) and in the IM-PegIFN (p=0.0887) arms respectively. Of interest, these results were confirmed when deaths with no evidence of AP-BC were considered as competing events. The TTP was still significant in the IM-400 arm (p=0.0002) and the IM-AraC arm (0.04). However, no TTP differences were observed in the IM-600 arm (p=0.38); moreover TTP is strictly similar in both molecular response rate groups at 3 months in the IM-PegIFN arm (p=0.96). We also analyzed the cumulative incidence of AP-BC within a period of 6 months according to the kinetic of molecular response (n=568 pts); 3 groups of pts were analyzed: ≤10% within 3 months, ≤10% within 6 months, still above 10% within 6 months. There is a significant advantage (p<0.0001) for early molecular response for all pts included in the trial except for those assigned to the IM-PegIFN arm (p=0.82). Of interest for pts assigned to the IM-PegIFN arm, rate of AP-BC were 3%, 4%, and 0% for the ≤10% within 3 months, ≤10% within 6 months, still above 10% within 6 months groups of pts, respectively. **Conclusion:** a 3-months BCR-ABL transcript below the level of 10% IS was associated with a PFS improvement. However, results which were observed with the addition of PegIFN or an increased dose of IM frontline do not confirm the relevance of the 10% BCR-ABL cut-off level as strong surrogate marker for progression to AP-BC. Pts assigned to the IM-PegIFN arm are at very low risk of progression to AP-BC even if their molecular response is delayed.

4 CML Therapy II [913-918]

[913] Impact of Balanced or Unbalanced Karyotype At Diagnosis On Prognosis of CML: Long-Term Observation From 1346 Patients of the Randomized CML Study IV. Fabarius, Mannheim. Introduction: Acquired genetic instability in chronic myeloid leukemia (CML) as a consequence of the translocation t(9;22)(q34;q11) and the resulting BCR-ABL fusion causes the continuous acquisition of additional chromosomal aberrations and mutations and thereby progression to accelerated phase (AP) and blast crisis (BC). At least 10% of patients in chronic phase (CP) CML show additional alterations at diagnosis. This proportion rises during the course of the disease up to 80% in BC. Acquisition of chromosomal changes during treatment is considered as a poor prognostic indicator, whereas the impact of chromosomal aberrations at diagnosis depends on their type. Patients with major route additional chromosomal alterations (major ACA: +8, i(17)(q10), +19, +der(22)t(9;22)(q34;q11) have a worse outcome whereas patients with minor route ACA show no difference in overall survival (OS) and progression-free survival (PFS) compared to patients with the standard translocation, a variant translocation or the loss of the Y chromosome (Fabarius et al., Blood 2011). However, the impact of balanced vs. unbalanced (gains or losses of chromosomes or chromosomal material) karyotypes at diagnosis on prognosis of CML is not clear yet. **Patients and methods:** Clinical and cytogenetic data of 1346 evaluable out of 1544 patients with Philadelphia and BCR-ABL positive CP CML randomized until December 2011 to the German CML-Study IV, a randomized 5-arm trial to optimize imatinib therapy by combination, or dose escalation and stem cell transplantation were investigated. There were 540 females (40%) and 806 males (60%). Median age was 53 years (range, 16-88). The impact of additional cytogenetic aberrations in combination with an unbalanced or balanced karyotype at diagnosis on time to complete cytogenetic and major molecular remission (CCR, MMR), PFS and OS was investigated. **Results:** At diagnosis 1174/1346 patients (87%) had the standard t(9;22)(q34;q11) only and 75 patients (6%) had a variant t(v;22). In 64 of 75 patients with t(v;22), only one further chromosome was involved in the translocation; In 8 patients two, in 2 patients three, and in one patient four further

chromosomes were involved. Ninety seven patients (7%) had additional cytogenetic aberrations. Of these, 44 patients (3%) lacked the Y chromosome (-Y) and 53 patients (4%) had major or minor ACA. Thirty six of the 53 patients (2.7%) had an unbalanced karyotype (including all patients with major route ACA and patients with other unbalanced alterations like -X, del(1)(q21), del(5)(q11q14), +10, t(15;17)(p10;p10), -21), and 17 (1.3%) a balanced karyotype with reciprocal translocations [e.g. t(1;21); t(2;16); t(3;12); t(4;6); t(5;8); t(15;20)]. After a median observation time of 5.6 years for patients with t(9;22), t(v;22), -Y, balanced and unbalanced karyotype with ACA median times to CCR were 1.05, 1.05, 1.03, 2.58 and 1.51 years, to MMR 1.31, 1.51, 1.65, 2.97 and 2.07 years. Time to CCR and MMR was longer in patients with balanced karyotypes (data statistically not significant). 5-year PFS was 89%, 78%, 87%, 94% and 69% and 5-year OS 91%, 87%, 89%, 100% and 73%, respectively. In CML patients with unbalanced karyotype PFS (p<0.001) and OS (p<0.001) were shorter than in patients with standard translocation (or balanced karyotype; p<0.04 and p<0.07, respectively). **Conclusion:** We conclude that the prognostic impact of additional cytogenetic alterations at diagnosis of CML is heterogeneous and consideration of their types may be important. Not only patients with major route ACA at diagnosis of CML but also patients with unbalanced karyotypes identify a group of patients with shorter PFS and OS as compared to all other patients. Therefore, different therapeutic options such as intensive therapy with the most potent tyrosine kinase inhibitors or stem cell transplantation are required.

914 Elevated Risk of Peripheral Artery Occlusive Disease (PAOD) in Nilotinib Treated Chronic Phase Chronic Myeloid Leukemia (CML) Patients Assessed by Ankle-Brachial-Index (ABI) and Duplex Ultrasonography

Michaela Schwarz, Berlin. In bcr-abl-positive CML first-line therapy with the second generation tyrosine kinase inhibitors (TKI) nilotinib or dasatinib results in superior response rates and prevention of transformation as compared to imatinib. Generally, these TKIs are associated with mild and reversible toxicities but recent reports have indicated an elevated risk of PAOD in patients (pts) treated with nilotinib (Aichberger et al. 2011, Tefferi et al. 2011, le Coutre et al. 2011, Quintás-Cardama et al. 2012). We therefore screened all 153 chronic phase CML pts at our center for PAOD by sequentially determining ABI, followed by duplex ultrasonography when indicated. Cardiovascular risk factors were assessed with a specific questionnaire and biochemical parameters associated with PAOD were analyzed. We here present a first interim analysis.

Overall, 146 of 153 pts were evaluable and categorized into five groups: (I) first-line imatinib (n = 53); (II) first-line nilotinib (n= 31); (III) second-line nilotinib (n = 32); (IV) previously exposed to nilotinib (n = 23) and (V) never treated with nilotinib and currently not on imatinib (n = 7).

A pathological ABI, defined as <0.9, occurred in 24/116 (21 %) of all examined patients, but was more frequent in pts on first-line (7/26; 27 %, p=0.0181) and second-line nilotinib (10/24; 42 %, p=0.0004) as compared to pts on first-line imatinib (3/45; 7 %) despite a significantly longer treatment on first-line imatinib (median 80.25 months, range 4 - 137) than on first-line nilotinib (median 25.48 months, range 5 - 49, p<0.001). Newly diagnosed PAOD defined as a peripheral vascular occlusive event or pathological duplex ultrasonography was observed in 13/146 (8.9 %) of all pts and was more frequent in pts on first-line nilotinib (3/31; 9.6%, p=0.1057), second-line nilotinib (5/32, 15.6 %, p=0.0166) or pts previously exposed to nilotinib (4/23; 17.4 %, p=0.0123) as compared to pts on first-line imatinib (1/53; 1.9 %). No substantial differences between the five groups with respect to clinical cardiovascular risk factors were observed. However, biochemical risk factor assessment showed significantly higher levels of cholesterol and LDL in pts receiving first-line (218.0 mg/dl, p< 0.001, and 135.5 mg/dl, p< 0.01) and second-line nilotinib (219.9 mg/dl, p < 0.001, and 138.9 mg/dl, p < 0.01) as compared to pts on first-line imatinib (167.2 mg/dl and 97.8 mg/dl).

Of 16 pts with PAOD under or after nilotinib treatment, including three additional patients from a second center, 4 pts received nilotinib as first-line, 10 pts as second-line and 2 pts as third-line treatment. Median age was 62.4 years (range, 38-76) and median duration of CML was 103.1 months (range: 22-209). Best responses observed in this group of patients were CMR or MMR in 10/16 (62.5 %) and CCyR or MCyR in 6/16 (37.5 %) of patients. Major cardiovascular risk factors such as hypertension (13/15; 87 %), diabetes mellitus (5/14; 36 %), nicotine abuse (11/15; 73 %), cholesterol (5/12; 42 %) or LDL (6/13; 46 %) elevation were detectable in the majority. Vascular lesions affected the lower limb in 15/16 (94 %) and the eye in 1/16 pts (6 %). Cardiovascular interventions included percutaneous transluminal angioplasty (PTA) (7/16; 44 %), stent-implantation (5/16; 31 %) and/or surgery, including amputation (5/16; 31 %). Nilotinib was discontinued in 10/16 pts (63 %), dose-reduced in 5/16 pts (32 %) and continued in 1/16 pts (6 %).

Conclusions: Prospective monitoring of pts with CML in chronic phase by sequential evaluation of ABI and duplex ultrasonography revealed a significantly higher frequency of PAOD in pts on nilotinib than in pts on imatinib. Mechanisms leading to the development of PAOD under nilotinib treatment remain unknown. Aside from elevation of cholesterol and glucose levels not yet fully understood mechanisms such as inhibition of targets involved in vascular cell homeostasis (i.e. DDR1, KIT or PDGFR) must be considered. More prospective data is needed to determine the cardiovascular risk attributable to nilotinib. But at present, caution is advised in pts with ≥ 2 major risk factors or a known history of arteriopathy.

[915] Efficacy and Safety of Ponatinib in Patients with Accelerated Phase or Blast Phase Chronic Myeloid Leukemia (AP-CML or BP-CML) or Philadelphia Chromosome-Positive Acute Lymphoblastic Leukemia (Ph+ ALL): 12-Month Follow-up of the PACE Trial. Kantarjian, Texas

Background: Many patients (pts) with advanced Ph+ leukemias experience failure of all currently available tyrosine kinase inhibitors (TKIs) targeting BCR-ABL and have limited treatment options. Ponatinib is a potent pan-BCR-ABL inhibitor that is active against native and mutated forms of BCR-ABL, including the TKI resistant T315I mutant. The efficacy and safety of ponatinib (45 mg orally once daily) in pts with AP-CML, BP-CML, or Ph+ ALL were evaluated in a phase 2, international, open-label clinical trial. **Methods:** The PACE trial enrolled 449 pts, including 85 AP-CML, 62 BP-CML, and 32 Ph+ ALL. Pts were resistant or intolerant (R/I) to dasatinib or nilotinib, or had the T315I mutation at baseline. AP-CML, BP-CML, and Ph+ ALL pts were assigned to 1 of 4 cohorts: AP-CML R/I, AP-CML T315I, BP-CML/Ph+ ALL R/I, BP-CML/Ph+ ALL T315I. Two AP-CML pts were not assigned to a cohort (post-imatinib, did not have T315I at baseline) and were excluded from efficacy analyses and included in safety analyses. The primary endpoint was major hematologic response (MaHR) at any time within 6 mos after treatment initiation. Data as of 23 July 2012 are reported, with a minimum follow-up of 9 mos (median 13 [4 to 21], 6 [0.1 to 18], and 6 [0.1 to 16] mos for AP-CML,

BP-CML, and Ph+ ALL, respectively). **Results:** The median age for AP-CML, BP-CML, and Ph+ ALL pts was 60, 53, and 62 yrs, respectively. Median time from initial disease diagnosis to start of ponatinib was 7, 4, and 1.5 yrs, respectively. Pts were heavily pretreated: 94% received prior imatinib, 88% dasatinib, 61% nilotinib; 8% received 1 prior approved TKI, 39% received 2, and 53% received 3. Sixteen percent had undergone prior stem cell transplant. In pts previously treated with dasatinib or nilotinib (N=171), 94% had a history of resistance to dasatinib or nilotinib, 6% were purely intolerant. Reported MaHR rates with the most recent dasatinib or nilotinib therapy were 35% AP-CML, 16% BP-CML, 43% Ph+ ALL.

At the time of analysis, 59% of AP-CML, 8% of BP-CML, and 9% of Ph+ ALL pts remained on study. Overall, the most common reasons for discontinuation were progressive disease (19%, 50%, and 53%, respectively) and adverse events (AEs; 11%, 16%, and 6%, respectively). Hematologic and cytogenetic response rates are shown in the table; MaHR and MCyR were observed across cohorts. MMR was achieved by 14% of AP-CML pts (14% R/I, 17% T315I). There was a trend for higher response rates among pts who received fewer prior approved TKIs. In AP-CML pts, the differences in MaHR rates by number of prior approved TKIs (1: 3/4 [75%]; 2: 20/33 [61%]; 3: 24/46 [52%]) were not significant (Fisher's Exact); differences in MCyR rates (1: 4/4 [100%]; 2: 13/33 [39%]; 3: 15/46 [33%]) were significant for pts treated with 1 vs 2 (p=0.0360) and 1 vs 3 prior approved TKIs (p=0.0168). Of pts achieving MaHR, 42% of AP-CML and 35% of BP-CML/Ph+ ALL pts were projected (Kaplan-Meier) to remain in MaHR at 1 yr. In AP-CML, the median progression-free survival (PFS) was estimated (Kaplan-Meier) as 80 (range 6 to 88) wks; the probability of maintaining PFS at 6 mos and 1 yr was estimated as 80% and 57%, respectively. Median overall survival (OS) had not yet been reached; the probability of OS at 6 mos and 1 yr was estimated (Kaplan-Meier) as 96% and 85%, respectively. In BP-CML/Ph+ ALL, median PFS was estimated as 18 (range 0.1 to 74) wks; the probability of maintaining PFS at 6 mos and 1 yr was estimated as 34% and 20%, respectively. Median OS was estimated as 30 (range 0.4 to 77) wks; the probability of OS at 6 mos and 1 yr was estimated as 54% and 34%, respectively. Ponatinib was generally well-tolerated; the most common treatment-related AEs were thrombocytopenia (29%), rash (25%), and neutropenia (22%). The most common serious treatment-related AEs were thrombocytopenia (3%) and pancreatitis (3%). Rash was generally grade 1 or 2 in severity. Thrombocytopenia, neutropenia, and pancreatitis were typically reported early in treatment and were manageable with dose modification.

Conclusions: Ponatinib was generally well-tolerated and had substantial activity in pts with AP-CML, BP-CML, or Ph+ ALL, regardless of mutation status or prior therapy. Data with a minimum follow-up of 12 mos will be presented.

	AP-CML		BP-CML		Ph+ ALL	
	R/I N=65	T315I N=18	R/I N=38	T315I N=24	R/I N=10	T315I N=22
MaHR ^a	58%	50%	32%	29%	50%	36%
MCyR	34%	56%	18%	29%	60%	41%
CCyR	22%	33%	16%	21%	50%	32%

^aBaseline MaHR counted as non-responder

[916] Discontinuation of Second Generation (2G) Tyrosine Kinase Inhibitors (TKI) in Chronic Phase (CP)-Chronic Myeloid Leukemia (CML) Patients with Stable Undetectable BCR-ABL Transcripts Rea, Paris, Background: TKI have proven remarkably successful against CML. Despite this progress, current recommendation is to never stop therapy. However, prospective trials such as STIM and CML8 suggest that imatinib may be stopped in patients with deep and sustained molecular responses (Mahon *et al*, Lancet Oncol. 2010; Ross *et al*, Haematologica 2012). Here, we report on the feasibility of 2G-TKI discontinuation. **Methods:** Adult CP-CML patients on dasatinib or nilotinib without previous allogeneic transplantation or progression to advanced phase CML were proposed treatment discontinuation provided that (1) TKI treatment duration was at least 36 months (2) BCR-ABL transcripts were undetectable for at least 24 months, with at least 20 000 amplified copies of the control gene. The primary objective was treatment-free stable major molecular response (MMR: BCR-ABL/ABL internationally standardized (IS) ratio ≤ 0.1% IS). BCR-ABL transcripts were quantified in local laboratories monthly during the first 12 months, every 2-3 months during the 2nd year and every 3-6 months thereafter. Molecular relapse was defined by the loss of MMR and triggered therapy resumption. The study is ongoing and 42 patients currently agreed to stop dasatinib (53%) or nilotinib (47%). Data as of August 1, 2012 are reported, focusing on the subgroup of 34 patients with a minimum follow-up of 6 months (median 14, range 7-33). **Results:** Median age was 58 (34-81), 56% of patients were females (n=19). Sokal risk group was low in 56% (n=19), intermediate in 24% (n=8), high in 11% (n=4) and unknown in 9% (n=3). Twelve patients (35%) received interferon prior to TKI therapy, 25 (74%) were treated with 2G-TKI upfront (n=2) or after imatinib intolerance (n=25) and 9 (26%) received 2G-TKI due to suboptimal response/resistance. The median time since diagnosis was 87 months (31-218), the median time since TKI initiation was 79 months (30-133), the median duration of 2G-TKI therapy was 35 months (21-72) and the median duration of undetectable BCR-ABL transcripts was 27 months (21-64). At last follow-up, the 12-month probability to remain in stable MMR was 58.3% (95% CI, 41.5%-75%). The median time to MMR loss was 4 months (1-25). Importantly, no hematologic relapse was observed and none of the patients progressed to advanced phase CML. Since in imatinib discontinuation trials, different definitions for molecular relapse were used, we also calculated the rate of relapse according to STIM (detectable BCR-ABL on 2 consecutive tests with at least 1 log increase between the 2) and CML8 (detectable BCR-ABL on 2 consecutive tests at any level). The corresponding 12-month probabilities were 55.8% (95% CI, 39.2%-72.6%) according to STIM and 44.1% (95%CI, 27.4%-60.8%) according to CML8. We searched for factors possibly associated with treatment-free stable MMR. Patients treated with 2G-TKI first line or after imatinib intolerance had a significantly higher probability of stable MMR than those who were switched to 2G-TKI because of suboptimal response/resistance (12-month rate 67.3% (95% CI, 48.6%-86%) versus 33% (95% CI; 2.5%-64.1%), p=0.02). Gender, age, prior IFN exposure, 2G-TKI type and treatment duration were not found to have any impact but caution is needed due to the small size and heterogeneity of this series. Treatment was resumed in 15 patients after a median time of 5 months (2-29). The same 2G-TKI used prior to discontinuation was reintroduced in all but 1 patient, due to tolerance issues. The median follow-up since treatment resumption was 9 months (0-

27). At last follow-up, MMR was regained in all 13/15 evaluable patients and undetectable *BCR-ABL* in 10/13. Eighteen patients with stable MMR remained off-therapy at last follow-up (median 16 months, range: 7-33), among which 7 with stable undetectable *BCR-ABL* and 11 with weakly detectable *BCR-ABL* on 1 or more occasions. **Conclusions:** 2G-TKI may be safely discontinued in CP-CML patients with long-lasting undetectable *BCR-ABL* under strict molecular monitoring conditions, especially in patients with prior imatinib intolerance or treated with 2G-TKI as first line therapy. In patients with prior imatinib suboptimal response/resistance, strategies to improve outcome are needed. The recurrence 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.

[917] Single Molecule Real Time (SMRT™) Sequencing Sensitive Detects the Evolution of Polyclonal and Compound BCR-ABL Mutations in Patients Who Relapse On Kinase Inhibitor Therapy Smith, San Francisco

Background: Secondary kinase domain (KD) mutations represent the most well-documented mechanism of resistance to tyrosine kinase inhibitors (TKIs) in chronic myeloid leukemia (CML). In CML, multiple TKIs with different mutation profiles are approved and the ability to detect KD mutations at the time of disease progression can impact therapy choice. To optimize clinical impact, second generation TKI selection must consider the majority TKI-resistant mutant population as well as smaller mutant sub-populations that may be selected with subsequent treatment. Sequential TKI therapy is associated with additional complexity: multiple mutations can coexist separately in an individual patient ("polyclonality") or can occur in tandem on a single allele ("compound mutations"). Multiple mutations are associated with poor clinical outcome (Parker et al., Blood 2012). Compound mutations can cause *in vitro* resistance to ponatinib, the only TKI clinically active against the highly resistant T315I mutation (Eide, et. al, ASH 2012 abstract #1416). Currently, no clinically adaptable technology can distinguish polyclonal from compound mutations. Due to the size of the *BCR-ABL* KD, most next-generation sequencing platforms cannot generate reads of sufficient length to determine if mutations separated by ≥ 500 nt reside on the same allele. Pacific Biosciences RS Single Molecule Real Time (SMRT) sequencing technology is a third generation deep sequencing technology capable of achieving average read lengths of ~ 1000 bp and frequently >3000 bp, enabling sensitive and accurate sequencing of the entire *ABL* KD on a single strand of DNA. Though allele-specific detection methods such as MassARRAY offer sensitivity as low as $\sim 0.5\%$, these assays are designed to detect a limited number (~ 31) of mutations whereas SMRT sequencing offers an unbiased approach capable of detecting novel variants. We sought to (1) develop a potential clinically-applicable SMRT sequencing assay for the detection of *BCR-ABL* KD mutations capable of distinguishing polyclonal and compound mutations, and (2) compare the accuracy and sensitivity of this method to standard sequencing and MassARRAY. **Results:** We assessed 54 samples from 36 CML patients who had clinically relapsed on *ABL* kinase inhibitor therapy and were previously analyzed by standard sequencing, and in a subset, by MassARRAY. We amplified an 863bp area of the *BCR-ABL* KD from patient-derived cDNA with primers containing 5' barcodes, enabling sequencing of 6 to 8 patient samples on a single SMRT cell on a single run. On average, 2519 reads were obtained for each sample per run (range 330 to 10,240). All of 131 known mutations detected by MassARRAY were identified by SMRT sequencing using a p-value threshold of $1.03e-03$. SMRT sequencing also identified all 107 known mutations detected by direct sequencing with a p-value threshold of $6.0e-08$. In addition to these known mutations, SMRT sequencing detected an additional 1320 non-silent mutations across all patient samples using a strict p-value threshold cut-off of $6e-08$, ranging in abundance from 0.2% to 17% (median 0.75%). Among 47 samples where >1 mutation was detectable by direct sequencing or MassARRAY, SMRT sequencing revealed that 40 (85%) had compound mutations detectable at a frequency of $\geq 1\%$. In total, we detected 73 different compound mutations at a frequency of $\geq 1\%$. In all cases where compound mutations were detected and more than one treatment timepoint was available, at least one compound mutation clearly evolved from a mutation detectable at a prior timepoint. In the most complex case, 4 separate mutations yielded 8 different mutant alleles. **Conclusions:** Pacific Biosciences RS SMRT sequencing detects KD mutations in patient samples with sensitivity comparable to or better than MassARRAY and can distinguish compound from polyclonal mutant clones. Among patient samples with multiple mutations, compound mutations were detectable in the vast majority of samples by SMRT sequencing, revealing a complex mutational landscape not demonstrable by other clinically viable sequencing methods and previously unappreciated. Given the growing numbers of patients exposed to multiple TKIs in a sequential manner, the ability to accurately and sensitively characterize drug-resistant alleles by SMRT sequencing promises to further facilitate a personalized approach to patient management and inform models of disease evolution.

[918] Natural History and Potential for Cure of Patients with Chronic Myeloid Leukemia in Chronic Phase Receiving Frontline Therapy with Recombinant Interferon-Alfa: 30-Year Update From M.D. Anderson Cancer Center. Quintás-Cardama, Texas.

Background: Prior to the advent of the tyrosine kinase inhibitor (TKI) imatinib, pioneering studies at our institution in the early 1980s established recombinant interferon-alfa (IFN- α) as standard therapy for chronic myeloid leukemia (CML). The use of IFN- α has come to the fore again given its therapeutic properties as an immunomodulatory agent and its putative activity against CML stem cells. We here provide an account of the natural history of patients with early chronic phase CML treated with IFN- α at our institution for the last 30 years. **Methods:** We analyzed 512 patients with early chronic-phase CML who were treated with IFN- α -based therapies between 1981-1995 for the rates of partial (PCyR) and complete cytogenetic response (CCyR), complete molecular response (CMR), major molecular response (MMR), overall survival (OS), transformation-free survival (TFS), and CML cure. **Results:** The median age of the cohort was 42 years (range, 15-76). The distribution of high, intermediate, and low risk patients by Sokal or Hasford was 21%/25%/36% and 10%/27%/44%, respectively. Of the 512 patients, 274 received IFN- α alone or in combination with hydroxyurea or high-dose chemotherapy, 148 received IFN- α and low-dose cytarabine, and 90 were treated with homoharringtonine followed by IFN- α as maintenance. After a median follow-up of 245 months (range, 4-360), the median OS was 82 months. The 5-, 10-, and 20-year survival was 62%, 41%, and 29%, respectively. Overall, 322 patients (63%) achieved a cytogenetic response, including CCyR in 140 (27%), which was obtained after a median of 16 months (range, 3-107 months), and PCyR in 72 (14%) for a major cytogenetic response rate of 41%. The median follow-up for patients who achieved CCyR was 252 months (range, 91-360). The 5-, 10-, and 20-year survival for patients who achieved CCyR was 90%, 79%, and 63%, respectively, with a 20-year TFS of 76%. Serial molecular monitoring by RT-PCR (at least 2 measurements) is available in 46 patients. Of them, 31 achieved CMR that lasted a median of 9 years (range, 1.5-17). Of them, 14 patients remain in CMR off therapy for

a median of 9.5 years (range, 1.5-17), 6 remain off therapy with detectable transcripts (5 in MMR) after a median of 10.5 years (range, 4.5-13), 9 remain in CMR after having relapsed and switched to other therapies (5 imatinib, 2 dasatinib, 1 allo-SCT, 1 chemotherapy), and 2 maintained MMR while receiving chemotherapy. Eight of the 31 patients relapsed (including 3 with sudden lymphoid BP). At the time of last follow-up, only 3 of the 31 patients who achieved CMR had died, one after 1 lymphoid BP, 1 acute myeloid leukemia (with deletion 7), and 1 myeloproliferative disorder (with trisomy 8). All patients eventually discontinued IFN- α therapy (192 resistance, 92 toxicity, 40 resistance/toxicity, BP 37, loss of CCyR 12, 3 death in CCyR, 100 lost to follow-up/other) and received subsequent therapy with TKIs (n=52), allo-SCT (n=68), other therapies (n=74), or unknown (n=314). One hundred twenty-seven patients are still alive and have been followed in our clinics at least once in the last 24 months. **Conclusion:** While currently superseded by imatinib and other TKIs, IFN- α remains an active agent in CML, capable of inducing CCyR in approximately 25% and CMR in 5%-7% of patients in CP. Most patients achieving CMR on IFN- α can safely discontinue therapy and remain in remission with no evidence of residual disease for more than 10 years, suggesting the possibility of CML cure. Some patients relapse molecularly but remain in "non-interventional CCyR" (i.e no therapy and detectable *BCR-ABL1* transcripts).

5 CML Therapy: Aiming for Deep Response [67-72]

[67] Complete Molecular Remission (CMR 4.5) of CML Is Induced Faster by Dose – Optimized Imatinib and Predicts Better Survival - Results From the Randomized CML-Study IV. Hehlmann, Germany. Dose optimized imatinib (IM) at doses of 400- 800mg has been shown to induce faster and deeper cytogenetic and molecular – responses than standard IM (400mg/day). Since complete molecular remission (CMR 4.5) identifies a subgroup of patients who may stay in remission even after discontinuation of treatment, it was of interest to analyse whether CMR 4.5 is reached faster with dose optimized IM and whether CMR 4.5 correlates with survival. CMR 4 and CMR 4.5 are defined as $\leq 0.01\%$ BCR-ABL IS or ≥ 4 log reduction and $\leq 0.0032\%$ BCR-ABL IS or ≥ 4.5 log reduction, respectively, from IRIS baseline as determined by real-time PCR. CML-Study IV is a five arm randomized study of IM 400 mg vs IM 400 mg + IFN vs. IM 400 mg + Ara C vs. IM after IFN failure vs. IM 800 mg. In the IM 800 arm, a 6 weeks run in period at IM 400 mg was followed by a dose increase to 800 mg and then by a dose reduction according to tolerability. Grade 3 or 4 adverse effects (AE) were to be avoided. From July 2002 to March 2012 a total of 1551 patients with newly diagnosed chronic phase CML were randomized of whom 1525 were evaluable. Median age was 52 years, 88% were EUTOS low risk, 12% high risk, 36% were Euro score low risk, 52% intermediate and 12% high risk, 38% were Sokal low risk, 38% intermediate and 24% high risk. 113 patients were transplanted, 246 received 2nd generation TKI. 152 patients have died, 90 of CML or unknown reasons, 62 of not directly CML-related causes. After a median observation time of 67,5 months 6 years OS was 88.2% and PFS 85.6%. CCR, MMR, CMR 4 and CMR 4,5 were achieved significantly faster with dose optimized IM (400 – 800 mg). No significant differences in remission rates were observed between IM 400 mg and the combination arms IM 400 mg + IFN and IM 400 mg + Ara C, whereas IM after IFN failure thus far yielded significantly slower response rates. After 4 years CCR rates were for IM 400, IM 400 + IFN, IM 400 + Ara C, IM 400 after IFN, and IM 800, 80%, 75%, 73%, 59% and 80%, respectively, MMR rates 84%, 77%, 82%, 61% and 88%, CMR 4 rates 57%, 55%, 55%, 40% and 65%, and CMR 4.5 rates 40%, 42%, 42%, 28% and 52%, respectively. CMR 4 was reached after a median of 27 months with IM 800 and 41.5 months with IM 400. CMR 4.5 was reached after a median of 41.5 months with IM 800 and 63 months with IM 400. EUTOS low risk patients reached all remissions faster than EUTOS high risk patients. The differences of CMR 4 rates between IM 800 and IM 400 at 3 years were 13% and at 4 years 8%, and of CMR 4.5 rates at 3 years 10% and at 4 years 13%. Grade 3 and 4 AE were not different between IM 400 and dose optimized IM 800. Independent of treatment approach, CMR 4 and more clearly CMR 4.5 at 3 years predicted better OS and PFS, if compared with patients without CMR 4 or CMR 4.5, respectively. CMR 4 and 4.5 were stable. After a median duration of CMR 4 of 3.7 years only 4 of 792 patients with CMR 4 have progressed. Life expectancy with CMR 4 and 4.5 was identical to that of the age matched population. We conclude that dose optimized IM induces CMR 4.5 faster than IM 400 and that CMR 4 and CMR 4.5 at 3 years are associated with a survival advantage. Dose optimized IM may provide an improved therapeutic basis for unmaintained treatment discontinuation in patients with CML. **NOTES.**

[68] Can the Combination of the Measurement of BCR-ABL1 Transcript Levels At 3 and 6 Months Improve the Prognostic Value of the 3 Month Measurement? Neelakantan, London. Several groups have shown that that the BCR-ABL1 transcript level measured at 3 or 6 months after starting TKI therapy strongly predicts for the achievement of cytogenetic and molecular responses and for PFS and OS. In particular, we have shown that CML patients treated with imatinib who at 3 months have a transcript level lower than 9.8% on the international scale or lower than 1.67% at 6 months fare significantly better. We have also shown that the molecular assessment made at 3 months on imatinib therapy is a better predictor of the prognosis of patients than the analysis of BCR-ABL1 transcripts at 6 months. Here we investigate whether it is possible to improve the prognostic accuracy of early measurement of the transcript level by combining the 3 and 6 month results. Between June 2000 and December 2010 282 consecutive adult patients with CML in CP seen at our institution received imatinib 400 mg daily as first line therapy. The median follow-up was 69 months (range 17-131). During follow-up 118 patients discontinued imatinib and received nilotinib (n=37), dasatinib (n=72) or an allogeneic stem cell transplant (n=9). BCR-ABL1 transcripts were measured in the blood at 6 to 12 week intervals using RQ-PCR and results were expressed as percent ratios relative to an ABL1 internal control with original laboratory values converted to the international scale. Two hundred and seventy-four patients were still alive in chronic phase and receiving imatinib at 6 months. We classified these patients according to their transcript levels at 3 months (lower or higher than 9.8%) and 6 months (lower or higher than 1.67%). 181 (66%) patients had low transcripts both at 3 and 6 months; these patients had an excellent outcome with a OS of 93.5% and a 100% cumulative incidence (CI) of CCyR. Fifty-seven (21%) of the patients had high transcript levels on both occasions; these patients had a significantly worse outcome than the previous cohort, namely an OS of 55.6% (p<0.001) and a CI of CCyR of 14.9% (p<0.001). Thirty (11%) patients had low transcript levels at 3 months but high transcript levels at 6 months; these patients had a prognosis similar to those of the patients with low transcripts at both the 3 and 6 month time points with an OS of 92.4 (p=0.78) and a 8-year CI of CCyR of 99.5% (p=0.001), although the

kinetics of the response in this cohort was slower. Only 6 patients (2%) had high transcript levels at 3 months but low levels at 6 months; these patients had an outcome similar to the patients with low transcript levels at the two time points (OS= 100%, PFS=83.3% and CI of CCyR = 85%). The measurement of the transcript level at 6 months adds very little prognostic discrimination to the measurement already taken at 3 months. The 11% of patients who met the three month milestone but failed the 6 month milestone had an OS and PFS identical to the patients who achieved both milestones. The CI of CCyR was also similar (although slower, median time to CCyR 12 months vs 6 months for the patients who met both milestones, p=0.001). The 2% of patients who failed the first milestone at 3 months but who met the second one at 6 months also seemed to fare well, although this group is too small to clearly establish whether patients with high 3 month transcript levels are 'rescued' by meeting the 6 month milestone. In summary, the prognosis of patients can be accurately established by assessing the transcript level purely at 3 months, although the analysis at 6 months may improve the prognostic classification of 2% of the patients.

[69] Assessment of Early Molecular Response As a Predictor of Long-Term Clinical Outcomes in the Phase 3 BELA Study. Brümmendorf, Achen.

Bosutinib (BOS) is an oral, dual Src/Abl kinase inhibitor. In the randomized, phase 3 BELA trial, BOS 500 mg/d demonstrated clinical activity in newly diagnosed (£6 mo) chronic phase chronic myeloid leukemia (CP CML) and manageable toxicity distinct from that of imatinib (IM) 400 mg/d. This analysis of the BELA trial investigated reduction in Bcr-Abl/Abl ratio at Months 3, 6, and 9 as early predictors of long-term outcomes. Baseline characteristics were well balanced between treatment arms. Dose escalation to 600 mg/d occurred in 6% of patients (pts) on BOS and 18% of pts on IM. The most common reason for BOS discontinuation was toxicity (24% vs 7% on IM), and for IM was progressive disease (13% vs 4% on BOS). Data included a minimum follow-up of 24 mo. In the intent-to-treat population, the rate of major molecular response (MMR; Bcr-Abl/Abl ratio £0.1% on International Scale [IS]) was higher at all time points for BOS versus IM, with a significantly shorter median time to MMR for BOS (48 wks) versus IM (73 wks; *P* <0.001). Among pts with molecular assessment at each time point, the rate of Bcr-Abl/Abl ratio £10% on the IS was significantly higher with BOS versus IM at Month 3 (86% vs 65%; *P* <0.001), Month 6 (94% vs 82%; *P* <0.001), and Month 9 (97% vs 90%; *P* = 0.01); a similar trend was observed for pts with Bcr-Abl/Abl ratio £1% at each time point (*Table*). In both arms, a Bcr-Abl/Abl ratio £10% versus >10% at Months 3, 6, and 9 was predictive of significantly higher cumulative rates of MMR and CCyR by both 12 mo and 24 mo (*Table*). The rates of MMR by 12 and 24 mo were generally highest among pts with a Bcr-Abl/Abl ratio £1% at Month 3, 6, and 9 in both arms. A similar trend was generally observed for on-treatment event-free survival (EFS; included death, transformation to accelerated/blast phase, increased white blood cell count without complete hematologic response [CHR], or loss of CHR or CCyR) and overall survival (OS) at 24 mo (*Table*). In the BOS arm, Kaplan-Meier estimates at 24 mo were significantly higher with a Bcr-Abl/Abl ratio £10% versus >10% for EFS at Months 3, 6, and 9, and for OS at Months 3 and 9. Furthermore, Kaplan-Meier estimates of EFS and OS at 24 mo were significantly higher for pts with Bcr-Abl/Abl ratio £10% versus >10% at Months 6 and 9 in the IM arm. Within each treatment arm, estimated rates of EFS and OS at 24 mo generally appeared similar for pts with reduction of Bcr-Abl/Abl ratio £1% versus £10% at early time points. In summary, a Bcr-Abl/Abl ratio £10% versus >10% at Months 3, 6, and 9 was associated with higher rates of MMR and CCyR by later time points in both arms. A lower Bcr-Abl/Abl ratio was also predictive of EFS after 24 mo of follow-up except for IM at Month 3, and of OS except for IM at Month 3 and BOS at Month 6. However, because of the very limited number of EFS and OS events and the relatively few pts with a Bcr-Abl/Abl ratio >10% (particularly in the BOS arm at Months 6 [n = 12] and 9 [n = 6]), longer follow-up in the BELA trial is needed to fully characterize the predictive properties of early reductions in Bcr-Abl/Abl ratio on long-term EFS and OS.

		Bcr-Abl/Abl ratio on IS at Month 3							
		BOS (n = 208)				IM (n = 223)			
		£1% (n = 81)	£10% ^a (n = 179)	>10% (n = 29)	<i>P</i> value ^b	£1% (n = 39)	£10% ^a (n = 146)	>10% (n = 77)	<i>P</i> value ^b
MMR									
	By 12 mo	82%	56%	17%	<0.001	72%	46%	5%	<0.001
	By 24 mo	91%	74%	21%	<0.001	85%	69%	17%	<0.001
CCyR									
	By 12 mo	100%	95%	48%	<0.001	92%	93%	55%	<0.001
	By 24 mo	100%	96%	48%	<0.001	92%	95%	65%	<0.001
	EFS at 24 mo ^c	96%	93%	83%	0.004	88%	92%	85%	0.331
	OS at 24 mo ^c	100%	99%	88%	0.004	97%	99%	95%	0.090
		Bcr-Abl/Abl ratio on IS at Month 6							
		BOS (n = 193)				IM (n = 226)			
		£1% (n = 157)	£10% ^a (n = 181)	>10% (n = 12)	<i>P</i> value ^b	£1% (n = 112)	£10% ^a (n = 186)	>10% (n = 40)	<i>P</i> value ^b
MMR									
	By 12 mo	67%	59%	0%	<0.001	60%	39%	0%	<0.001
	By 24 mo	82%	76%	8%	<0.001	84%	61%	8%	<0.001
CCyR									
	By 12 mo	98%	97%	33%	<0.001	98%	90%	30%	<0.001
	By 24 mo	99%	98%	33%	<0.001	98%	93%	48%	<0.001
	EFS at 24 mo ^c	94%	94%	64%	0.003	97%	94%	67%	<0.001
	OS at 24 mo ^c	99%	99%	92%	0.096	99%	98%	90%	0.014
		Bcr-Abl/Abl ratio on IS at Month 9							
		BOS (n = 185)				IM (n = 216)			

	£1% (n = 160)	£10% ^a (n = 179)	>10% (n = 6)	P value ^b	£1% (n = 134)	£10% ^a (n = 195)	>10% (n = 21)	P value ^b
MMR								
By 12 mo	65%	59%	0%	0.004	50%	35%	0%	<0.001
By 24 mo	84%	77%	0%	<0.001	78%	59%	5%	<0.001
CCyR								
By 12 mo	98%	97%	17%	<0.001	98%	89%	29%	<0.001
By 24 mo	100%	99%	17%	<0.001	99%	94%	43%	<0.001
EFS at 24 mo ^c	95%	95%	0%	<0.001	96%	95%	49%	<0.001
OS at 24 mo ^c	100%	99%	83%	0.001	99%	99%	85%	<0.001

^aIncludes pts with Bcr-Abl/Abl ratio £1%.

^bComparison of pts with Bcr-Abl/Abl ratio £10% vs >10% on the IS.

^cBased on Kaplan-Meier estimates.

[70] Early Molecular and Cytogenetic Responses Predicts for Significantly Longer Event Free Survival (EFS) and Overall Survival (OS) in Patients (pts) with Newly Diagnosed Chronic Myeloid Leukemia (CML) in Chronic Phase (CP) – an Analysis of 4 Tyrosine Kinase Inhibitor (TKI) Modalities (standard dose imatinib, high dose imatinib, dasatinib and nilotinib). Jain, Houston. Introduction: Early responses to TKI are an important predictor of long term outcome. Several approaches have been used to improve outcomes in CML-CP, including high-dose imatinib, and 2nd generation TKI. These induce earlier responses and improved long-term outcomes. We analyzed patterns of response and their long-term impact among pts treated with 4 TKI modalities as frontline CML CP therapy. **Methods:** 489 CML CP pts (median age 48 yrs, range 15-86 yrs) receiving initial therapy with TKI at MDACC from 2000 to 2011 were included in the analysis (missing values were excluded from the analysis). Patients received imatinib 400 mg/d (IM400; n=83), imatinib 800 mg/d (IM800; n=199), nilotinib (n=105) or dasatinib (n=102) in consecutive or parallel trials. Median follow-up was 76.5 months (mo) (3-136). Cytogenetic (G-banding) and molecular responses (real-time PCR, expressed in international scale) were assessed every 3 mo for the 1st year and every 6 mo thereafter. **Results:** After 3 mo of treatment, 301 (65%) patients achieved a cytogenetic response (Ph+ ≤ 0%) 105 (23%) achieved (Ph+ ≤ 1-35 %) and 52 (11 %) (Ph+ >35 %). Molecular response (≤ 1%) was achieved in 300 pts (79%) at 3 mo, while 66 (17%) achieved BCR-ABL 1-10 % and 13 pts (3%) had poorer molecular response (>10%) at 3 mo. Disease transformation was observed in 10 pts (2%), events observed were 54 (11%) and 62 pts (12%) died. Landmark analysis at 3-mo by molecular and cytogenetic response showed that molecular response (≤1, 1-10 and >10 %) after 3 mo of TKI did not discriminate for 3-year OS; cumulative proportions at 3 yr OS among each category were [BCR-ABL ≤1 % (97%), 1-10% (98%) and >10% (100%), p=0.593 by log rank test]. While cytogenetic responses (≤ 0 %, 1-35 and >35 %) after 3 mo of TKI significantly discriminated for 3-year OS [Ph+ ≤ 0% (98%), Ph+ 1-35% (95%) and Ph+ >35% (87%), p=0.002]. The 3-yr EFS in cumulative proportions by molecular response at 3 mo were (95 %) for BCR-ABL ≤1, (98%) if 1-10% and (64%) if >10% (p=0.001). Corresponding values for EFS by cytogenetic responses at 3 mo were: (97%) for Ph+ ≤ 0%, (88%) for Ph+ 1-35% and (85%) for Ph+ >35% (p=0.001). TFS 3-yr cumulative proportions for BCR-ABL ≤1% were (99%), for 1-10% it was (98%) and for >10% it was (100%) (p=0.87); corresponding rates by cytogenetic response were (99%) for Ph+ ≤ 0%, (97%) for Ph+ 1-35% and (94%) for Ph+ >35% (p=0.05). Similar results were obtained analyzing OS, EFS and TFS by the same molecular and cytogenetic response categories at 6 mo, except that the molecular response significantly predicted for a better 3 year OS at 6 month landmark analysis (p=0.02). There were no statistically significant differences in OS, EFS and TFS for pts achieving equivalent responses with different treatment modalities. However, pts treated with dasatinib or nilotinib, and to some extent IM800 had a significantly higher probability of achieving the deepest 3-mo response. (Table 1 below). **Conclusions:** The achievement of early (3 and 6 mo) molecular and cytogenetic responses with TKI is predictive for long term EFS, TFS and OS. The impact of the response is similar regardless of TKI used. However, pts treated with dasatinib and nilotinib, and to some extent those treated with high-dose imatinib, have a better probability of achieving the deepest responses at early time points.

Table -1- Landmark analysis at 3 and 6 months by molecular and cytogenetic response for predicted EFS, OS and TFS in response to each TKI therapy.

Landmark = 3 & 6 mo survival		% with response / % 3-y EFS / % 3-y OS/ % 3-y TFS			
		IM400	IM800	Nilotinib	Dasatinib
3 mo Cyto Res	0	34/92/100/96	62/97/97/99	81.3/97/97/99	84/99/98/100
	35<0	32/88/88/96	29/88/96/98	13/92/100/92	13/91/100/100
	>35	34/89/77/96	9/83/100/88	5.2/67/100/100	3/67/100/100
3 mo Mol Res	≤1	71/82/100/92	78/98/97/99	82/96/97/99	79/100/98/100
	>1 - 10	23/100/100/100	19/100/96/100	17/94/100/94	15/100/100/100
	>10	6/100/100/100	3/80/100/100	1/100/100/100	6/27/100/100
6 mo	0	41/97/100/97	85/97/99/98	97/99/100/99	91/99/99/100
	35<0	37/88/96/94	11/80/95/100	0/NA/NA/NA	6/75/100/100

Cyto Res	>35	23/82/59/95	4/83/86/100	3/67/100/67	3/100/100/100
6 mo Mol Res	≤1	60/67/99/100	89/96/99/98	91/99/100/99	89/100/100/100
	>1 - 10	40/100/100/100	9.7/79/94/100	6/100/100/100	8/42/100/100
	>10	0/NA/NA/NA	1/100/100/100	3/67/100/67	3/100/100/100

NA = no data available

[71] Nomogram to Prognosticate Outcomes in Patients with Chronic Myeloid Leukemia At Any Time-Point During Tyrosine Kinase Inhibitor Therapy. Quintás-Cardama, Houston. Background: The NCCN and the European LeukemiaNet guidelines for monitoring patients with chronic myeloid leukemia in chronic phase (CML-CP) provide recommendations for response assessment and treatment at 3, 6, 12, and 18 months based on evidence obtained in clinical trials. A clear limitation of such guidelines is their applicability at time-points different from those pre-specified. To overcome these limitations we have developed a novel statistical approach to CML prognostication. **Method:** In order to build our prognostic model, we used two cohorts of patients with CML-CP treated in the frontline DASISION phase III study (CA180-056) and the cohort of patients treated after imatinib failure in the dasatinib dose-optimization phase III study (CA180-034). Progression-free survival (PFS) was defined as any of the following: doubling of white cell count to $>20 \times 10^9/L$ in the absence of complete hematologic response (CHR); loss of CHR; increase in Ph+ BM metaphases to $>35\%$; transformation to AP/BP; or death. A modified Cox proportional hazards model was used to build a prognostic nomogram. **Results:** A total of 1189 patients were used for this analysis: 519 from DASISION (259 dasatinib and 260 imatinib) and 670 from CA180-034. First, we devised a model to link a BCR-ABL1/ABL1 ratios (according to the International Standard) obtained at specific time points during the course of treatment with patients' outcomes (PFS). For instance, at 18 months after front-line treatment, the future PFS probabilities are shown in Figure 1A. At 6 months after second-line treatment, the future PFS probabilities are shown in Figure 1B. Once the model was validated at specific time points, we next designed a nomogram to calculate patients' outcomes at any time point during the course of therapy by plotting 'master PFS curves' derived from the patient cohorts according to time. Figure 2A&B give the 90% quantile of the remaining PFS for patients at any time after front-line and second-line treatment, respectively. These may be used as a guideline for considering other treatment options when patients' BCR-ABL1/ABL1 ratios exceed these values. Figure 2 shows that the remaining PFS times for either front- or second-line treated patients depend mostly on the current BCR-ABL/ABL ratio and less on the time at which the ratio is obtained, reflected by the fact that the curves showing future PFS probabilities are characterized by smooth slopes. Figure 2A shows that 10% of front-line treated patients whose BCR-ABL1/ABL1 ratios are 50% or higher will have remaining PFS times of less than 12 months. If BCR-ABL1/ABL1 ratios are 75% or higher, then 10% of them will have remaining PFS times of less than 6 months. Similarly, Figure 2B shows that for second-line treated patients whose BCR-ABL1/ABL1 ratios are 50% or higher, 10% of them will have remaining PFS time shorter than 6 months. **Conclusion:** We have designed a nomogram that predicts PFS for patients treated in the frontline and second line settings according to their BCR-ABL1/ABL1 ratios, independent from the time at which these ratios are obtained. A similar approach has been taken to predict failure-free and overall survival and will be presented at the meeting. This prognostic tool is readily available for clinical purposes and might greatly facilitate monitoring and prognostication in CML.

[72] The Clinical Impact of Time to Response in De Novo Accelerated Phase Chronic Myeloid Leukemia (CML-AP) Ohanian, Houston. Background: Early (3-month) response is important in chronic phase chronic myeloid leukemia (CML-CP). Whether this applies also to CML in accelerated phase (CML-AP) has not been analyzed. **Aim:** To describe the impact of time to response on the outcome of patients (pts) with CML-AP. **Methods:** Frontline tyrosine kinase inhibitor (TKI) therapy was administered on consecutive or parallel clinical trials to 58 CML pts presenting with features of AP at the time of diagnosis, defined as blasts $\geq 15\%$ (n=8), basophils $\geq 20\%$, (n=22), platelets $<100 \times 10^9/L$ (n=3), cytogenetic clonal evolution (n=22), or more than 1 feature (n=3). 36 pts received imatinib. 22 pts received a 2nd generation TKI (2GTKI) (dasatinib, n = 5 or nilotinib, n = 17). We analyzed the impact of various degrees of molecular and cytogenetic responses at 3, 6, and 12-months on rates of major molecular response (MMR), MR^{4.5}, overall survival (OS), event free survival (EFS), and transformation (to blast phase) free survival (TFS). **Results:** After a median follow-up of 73 months (range 3 to 142) the overall rate of CCyR was 81% (imatinib 75%, 2GTKI 91%), and MMR 69% (imatinib 64%, 2GTKI 77%). At 3 yrs, TFS was 92%, EFS 90% and OS 87%. At 3 months 43 (81%) out of 53 evaluable pts had achieved a major cytogenetic response (MCyR) (71% of pts treated with imatinib, 95% of pts treated with 2GTKI); BCR-ABL/ABL $<10\%$ was achieved in 27 (96%) out of 28 evaluable pts (100% with imatinib, 95% with 2GTKI). At 6 months, 44 (86%) out of 51 evaluable pts had achieved a MCyR (83% of pts treated with imatinib, 90% of pts treated with 2GTKI); BCR-ABL/ABL $<10\%$ was achieved in 26 (93%) out of 28 evaluable pts (100% with imatinib, 90% with 2GTKI). The probability of achieving various outcomes: MMR, MR^{4.5}, OS, EFS, and TFS by response at 3 and 6 months are shown in Table 1 and 2. The number of pts evaluable for both cytogenetic and molecular responses at 3 and 6 months were 44 and 45 respectively. **Conclusion:** Pts with de novo CML-AP have an excellent outcome with TKIs, particularly 2GTKIs. As for chronic phase, pts with deep responses at 3 months (MCyR or BCR-ABL $<10\%$) have the best probability of a favorable long-term outcome, although a few have not achieved MCyR at 3 months. TKIs should be standard for all pts with AP features at the time of diagnosis.

Table 1. Outcome probability at 36 months by response at 3 months for pts evaluable by both molecular and cytogenetic responses

	Ph $\leq 35\%$	Ph $>35\%$
No.	41	3

MMR %	88 (n=36)	33 (n=1)
MR ^{4,5} %	66 (n=27)	33 (n=1)
TFS %	97	67
EFS %	94	67
OS %	97	67

Table 2. Outcome probability at 36 months by response at 6 months for pts evaluable by both molecular and cytogenetic responses

	Ph ≤35%	Ph >35%
No.	40	5
MMR %	90 (n=36)	40 (n=2)
MR ^{4,5} %	68 (n=27)	40 (n=2)
TFS %	97	100
EFS %	97	75
OS %	100	75

6 CML Therapy: Pharmacodynamic Markers and Novel Treatment Approaches [691-696]

[691] Relative Increase of Lymphocytes As Early As 1 Month After Initiation of Dasatinib Is a Reliable Predictor for Achieving Complete Molecular Response At 12 Months in Chronic Phase CML Patients Treated with Dasatinib.

Matsuki, Tokyo. Background: Tyrosine kinase inhibitor (TKI) therapy has become the standard treatment for patients with chronic phase chronic myelogenous leukemia (CML-CP). Compared with imatinib, dasatinib is known to induce a faster and better response as a first- and second-line treatment. A unique effect of dasatinib treatment is the induction of clonal lymphocytosis with large granular lymphocyte (LGL) morphology, presumed to be the effect of off-target kinase inhibition. These LGLs are known to possess either cytotoxic T-cell (CTL) or natural-killer (NK) cell phenotype. Several reports have shown that this clonal LGL expansion is associated with a better response to dasatinib treatment. **Purpose:** To prospectively analyze the immunoprofile of Japanese patients treated with dasatinib, and to correlate the increase of lymphocytes and their subsets (LGL, CTL and NK cells) with the clinical efficacy of dasatinib. **Method and Patients:** Japanese patients with CML-CP who were resistant or intolerant to first-line imatinib therapy participated in the prospective phase II study assessing the efficacy and safety of dasatinib. Peripheral blood LGL, CTL and NK cell counts before and 2 weeks, 1 month (M), 3M, and 6M after the initiation of treatment were analyzed at the central laboratory (BML Inc.) by flow cytometry. LGL, CTL and NK cells were defined as CD57+/CD14-, CD8+/CD4- and CD56+/CD3- cells, respectively. Lymphocytosis was defined as a lymphocyte count of >3.0x10⁹/L. The clinical efficacy of dasatinib was evaluated at 12M as complete molecular response (CMR) or less than CMR by peripheral blood quantitative RT-PCR (BML Inc.). The full data set was available for 50 out of 65 patients and was included in this analysis. **Results:** The median age of the patients enrolled was 57 years (range: 16 – 87 years). There were 37 male and 13 female patients. Twenty patients were switched to dasatinib due to intolerance to imatinib and 30 due to resistance. Lymphocytosis was observed in 19 patients (38%) at 3M. Of those 19 patients, 14 (73.7%) had lymphocytosis persisting at 6M. In total, 21 patients (42%) achieved CMR at 12M. The absolute count of lymphocytes, LGL, CTL or NK cells, did not differ significantly between patients who achieved CMR at 12M and those who did not (p>0.05). However, there were significant differences between these two groups in the relative increase of these cell counts. Relative increase of lymphocytes compared to baseline as early as 2 weeks after initiation of treatment was identified by univariate analysis as a significant factor associated with CMR at 12M (p=0.0348). This increase persisted at each time point observed until 6M. As for the different subsets of lymphocytes, the relative increase of CTL at 3 and 6M and LGL at 1, 3 and 6M were also significantly associated with a higher rate of CMR at 12M. When adjusted for possible confounding factors (age, sex, performance status, whether 1st or 2nd CP, resistance or intolerance to imatinib), the relative increase of lymphocytes at 1, 3 and 6M, CTL and LGL at 3 and 6M remained significant (Fig a-c). Patients who showed an increase of lymphocytes of over 1.5-fold at 1M showed a significantly higher rate of CMR at 12M (66.7% vs 25.8%, p=0.0007). Similar differences were seen in patients with increases of over 2-fold in CTL, LGL and NK cells (70.6% vs 22.6%, p=0.0011; 74% vs 21%, p=0.0003; and 57.9% vs 28.6%, p=0.0444). Detailed analysis at 3M showed a further significant difference in the treatment effect. An increase of over 2-fold in lymphocytes (81.3% vs 21.2%, p<0.0001), and of over 5-fold in CTL (85.7% vs 30%, p<0.0001), LGL (66.7% vs 30.3%, p=0.0001) and NK cells (75% vs 31.6%, p<0.0001) compared to baseline was associated with a significantly increased probability of CMR at 12M. **Conclusion:** Lymphocytosis after the initiation of dasatinib was seen in a substantial proportion of Japanese patients treated with dasatinib. A relative increase of lymphocytes together with LGL, CTL and NK cells, uniformly defined by flow cytometry, at 1 and 3M after the initiation of treatment were associated with a higher rate of CMR at 12M. To our knowledge, this is the first large prospective study analyzing the clinical significance of lymphocytosis associated with dasatinib therapy in Japanese patients with CML-CP using flow cytometry. Our results suggest that a relative increase of lymphocytes as early as 1M after the initiation of dasatinib is a reliable predictive marker for achieving CMR at 12M.

[692] Dissecting the Complexity of Philadelphia-Positive Mutated Populations by Ultra-Deep Sequencing of the Bcr-Abl Kinase Domain: Biological and Clinical Implications. Soverini, Bologna. Background and Aims – In chronic myeloid leukemia (CML) and Philadelphia-positive acute lymphoblastic leukemia (ALL), tyrosine kinase inhibitor (TKI) therapy may select for drug-resistant Bcr-Abl mutants. Mutation status of resistant patients is usually investigated by Sanger sequencing (SS) of the Bcr-Abl kinase domain (KD). Novel ultra-deep sequencing (UDS) technologies allow to conjugate higher sensitivity with the unprecedented possibility to perform instant cloning of thousands of DNA molecules. We thus decided to take advantage of an UDS-based approach in order to: 1) resolve qualitatively and quantitatively the complexity of mutated populations surviving TKIs; 2) investigate their clonal structure and evolution in relation to time and treatment.

Methods – We retrospectively performed a longitudinal analysis of a total of 111 samples from 35 CML or Ph+ ALL patients who had received sequential treatment with multiple TKIs (two to four TKIs among imatinib, dasatinib, nilotinib, ponatinib) and had experienced sequential relapses accompanied by selection of TKI-resistant mutations. All samples had already been scored by SS; 74/111 (67%) were positive for one (n=33) or multiple (n=41) mutations. UDS of the Bcr-Abl KD was done using Roche 454 technology. UDS allowed to achieve a lower detection limit of at least 0.1% – as compared to 20% of SS. **Results** – Bcr-Abl KD mutation status was found to be more complex than SS had previously shown in 85/111 (77%) samples (representative examples are detailed in Table 1). In 33/74 (44%) samples known to harbour one or more mutations by SS, UDS revealed that up to four ‘minor’ mutations with 1-20% abundance were present in addition to the ‘dominant’ one(s). The type of mutations could easily be accounted for by TKI exposure history, since the majority were known to be poorly sensitive either to the current or to the previous TKI received. The higher degree of complexity was evident also when the clonal relationships of multiple mutations were reconstructed (Table 1). This revealed that identical mutations may be acquired in parallel by independent populations (e.g., one wild-type and one already harboring a mutation), via the same or different nucleotide changes leading to the same amino acid substitution (convergent evolution). In addition, longitudinal quantitative follow-up of mutated populations revealed that:

- 1) complexity generally increases with increasing lines of TKI therapy;
- 2) with a few exceptions, double compound mutants have higher selective advantage over single mutants but also over triple;
- 3) however, whether a compound mutant will ultimately take over depends on TKI, treatment duration, competition with other coexisting populations - the same compound mutants behaved differently in different patients receiving the same TKI.

Conclusions – 1) sequential changes in the selective pressure exerted by TKIs may result in a heterogeneous mosaic of subclones harbouring different mutations or mutation combinations;

- 2) the evolution of each subclone is shaped not only by its inherent sensitivity to the specific TKI administered (‘absolute’ fitness) but also by the competition with all other coexisting subclones (‘relative’ fitness); (nonlinear) acquisition of additional mutations dictates further dynamics of shrinkage/expansion over time;
- 3) information provided by SS may not always be sufficient to predict responsiveness to a TKI;
- 4) sensitivity of a single or a compound mutant to a TKI *in vivo* is dictated by more complex factors than the mere *in vitro* IC50 value.

[693] Early Molecular Response to Imatinib in CP-CML Patients: The Significance of Early Dose Intensity and OCT-1 Activity in Responders and Efficacy of Dose Escalation and Switch to Nilotinib in Non-Responders. White, Adelaide.

Background: Accurate and early response prediction in CML patients treated with imatinib (IM) will facilitate timely therapeutic intervention in those patients predicted to respond poorly. The response of CP-CML patients to imatinib can be predicted by the BCR-ABL level at 3 months (early molecular response – EMR) with a level of $\geq 10\%$ (IS) (EMR failure) associated with inferior outcomes. Response can also be predicted by OCT-1 activity (OA); a measure of active transport of IM into target leukemic cells. In the ALLG TIDEL II study patients were treated with 600mg of IM upfront, and dose escalate (800mg) if IM blood levels are $<1000\text{ng/ml}$ at day 21. Patients were also dose escalated or switched to nilotinib for failure to achieve, or loss of, specified time dependent molecular milestones, including EMR failure. The aims of this study were to (1) assess the predictive value of EMR (2) assess the impact of factors which likely contribute to EMR such as dose intensity and OA, and (3) assess factors that modify the risk profile within the cohort who achieve EMR. **Results:** 210 patients were enrolled to TIDEL II. For this analysis 200 patients were analysed based on the availability of complete OA, day 21 blood levels and 3 month BCR-ABL% data. EMR was associated with a significantly higher rate of major molecular response (MMR) compared to that of patients with EMR failure (n=25/200) at both 12 (70% vs 20% $p<0.001$) and 24 months (82% vs 24% $p<0.001$), and a significantly higher event free survival (EFS)[^] (83% vs 60%, $p=0.013$). Similarly, patients with high OA achieved MMR at a higher rate than those with low OA (12 mo 73% vs 53% $p=0.003$ and 24 mo 82% vs 66% $p<0.001$), and had significantly higher EFS (89% vs 71% $p=0.011$). IM drug levels at day 21 were significantly lower in patients with EMR failure (median 1050 vs 1620ng/ml: $p=0.004$). Levels $<1000\text{ng/ml}$ were associated with a significantly higher rate of EMR failure (26% compared with 9% if levels $>1000\text{ng/ml}$ $p<0.001$). Importantly, the negative impact of low IM levels was confined to patients with low OA. EMR failure was no different in patients with high OA regardless of drug level (17% vs 9% $p>0.05$), whereas 35% of patients with low OA and IM levels $<1000\text{ng/ml}$ had EMR failure compared to 11% in patients with low OA and satisfactory drug levels. ($p=0.008$). This suggests that IM dose and the intracellular level of drug achieved (OA) are key determinants of the 3 month response. 22/25 patients with EMR failure remained on study at 6 months, 21 of whom were either dose increased or switched to nilotinib. Four of 8 patients with EMR failure and high OA achieved 1% BCR-ABL (IS) by 6 months, compared to 1/14 (7%) of patients with low OA and EMR failure. This suggests that early intervention in patients with EMR failure may not be effective in patients with low OA. Interestingly, the risk of events for patients with EMR failure, compared to those who achieve EMR but have low OA was equally high (EFS; 60% v 74% $p=0.280$). Importantly, 5/6 (83%) patients who transformed had low OA however 3/5 had achieved EMR. Similarly, the risk of mutation development was significantly associated with low OA, and not predicted by EMR (Table 1). While no patient with EMR failure achieved MR4.5 by 24 months, there was a significant difference in the proportion of patients achieving MR4.5, based on OA, within the cohort of patients who achieve EMR ($p=0.003$) (Table 1). **Conclusion:** Despite early intervention to increase dose intensity in cases of low drug levels or EMR failure (dose escalation and/or switch to nilotinib), OA, drug levels, and EMR all remain strong predictors of outcome. Importantly the achievement of EMR does not represent a safe haven for patients with low OA. These data suggest that optimising outcomes in high-risk CML cases may not be achievable even with early proactive intervention. Hence, the use of OA and other potential biomarkers at diagnosis may better identify patients with high-risk CML in whom therapy, other than currently available TKIs, may be required.

[694] Switching to Nilotinib Is Associated with Continued Deeper Molecular Responses in CML-CP Patients with Minimal Residual Disease After ≥ 2 Years On Imatinib: Enestcmr 2-Year Follow-up Results. Hughes, Adelaide.

Background: Superior rates of deeper molecular responses were achieved with nilotinib vs imatinib in patients newly

diagnosed with Philadelphia chromosome–positive (Ph+) chronic myeloid leukemia in chronic phase (CML-CP) in the Evaluating Nilotinib Efficacy and Safety in Clinical Trials—newly diagnosed patients (ENESTnd) trial. In addition, the 12-month (mo) analysis of the ENEST—complete molecular response (ENESTcmr) study demonstrated that switching to nilotinib after a minimum of 2 years on imatinib led to increased rates of major molecular response (MMR) and deeper molecular responses vs remaining on imatinib. Results from ENESTcmr are presented here with minimum 24 mo of patient follow-up. **Methods:** Patients with Ph+ CML-CP who had achieved complete cytogenetic responses but still had persistent BCR-ABL positivity by real-time quantitative polymerase chain reaction (RQ-PCR) after ≥ 2 years on imatinib were eligible. Patients (n = 207) were randomized to switch to nilotinib 400 mg twice daily (BID; n = 104) or to continue on the same dose of imatinib (400 or 600 mg once daily [QD]; n = 103). Rates of MMR, MR4 (BCR-ABL $\leq 0.01\%$ according to the International Scale [IS], corresponding to a 4-log reduction), MR4.5 (BCR-ABL $\leq 0.0032\%$ IS, corresponding to 4.5-log reduction), and undetectable BCR-ABL via RQ-PCR with ≥ 4.5 -log sensitivity were measured. **Results:** Among all randomized patients (intent-to-treat population), significantly more patients treated with nilotinib continued to achieve undetectable BCR-ABL by 24 mo (32.7% on nilotinib vs 16.5% on imatinib; $P = .005$; Table). The difference between the arms in achievement of this endpoint increased between 1 and 2 years (from 12.4% to 16.2%). The median time to MR4.5 and undetectable BCR-ABL was also significantly faster on nilotinib than on imatinib ($P = .005$ and $.003$, respectively). Cumulative rates of MR4.5 and undetectable BCR-ABL continued to be higher with nilotinib in patients without those responses at baseline, and the difference between arms appeared to increase over time. The safety profiles for nilotinib and imatinib were consistent with prior studies. By 24 mo, no patients in either arm progressed to accelerated phase/blast crisis. No patients on nilotinib died since the 12-mo analysis; 1 patient on imatinib died from metastatic prostate cancer in follow-up after discontinuation from the study. **Conclusions:** Switching to nilotinib led to significantly faster, deeper molecular responses in patients with minimal residual disease on long-term imatinib therapy. Since the 12-mo analysis, rates of deep molecular response (MR4.5 and undetectable BCR-ABL) have remained significantly higher in patients who did not have the response at baseline and were switched to nilotinib (vs those remaining on imatinib). In fact, the difference in favor of nilotinib increased between 1 and 2 years. These results suggest that switching to the more potent, selective tyrosine kinase inhibitor nilotinib is beneficial in patients with minimal residual disease after long-term imatinib therapy. Achievement of these deeper molecular responses (MR4.5 and undetectable BCR-ABL) after switching to nilotinib may enable a greater proportion of CML-CP patients to be eligible for future discontinuation studies. Cumulative rates of confirmed undetectable BCR-ABL by 24 mo will be presented as the confirmation assessments for several responders were not available at the time of this analysis.

[695] Efficacy and Safety of Radotinib in Chronic Phase Chronic Myeloid Leukemia Patients with Resistance or Intolerance to BCR-ABL Tyrosine Kinase Inhibitors: Radotinib Phase 2 Clinical Trial . Kim, Busan. Background Radotinib is a novel, selective Bcr-Abl tyrosine kinase inhibitor (TKI) developed by IL-YANG Pharm, South Korea. Radotinib showed a good efficacy and safety profile to chronic myeloid leukemia (CML) in preclinical and phase 1 clinical studies. To investigate the clinical efficacy and safety of radotinib 400 mg twice daily, data from CML patients treated during phase 2 clinical trial are reported. **Methods** Philadelphia chromosome (Ph+)-positive chronic phase CML (CP-CML) patients who failed or were intolerable to TKIs (imatinib and/or dasatinib and/or nilotinib) were enrolled between July 2009 and November 2011. Patients were treated with radotinib 400 mg twice daily for 12 cycles (1 cycle=4 weeks). The primary end point was an achievement of major cytogenetic response (MCyR, Ph+ $\leq 35\%$) by 12 months. Safety parameters were also analyzed. **Results** A total of 77 CP CML patients (18 years of age or over) were enrolled from 12 sites in Korea, India, and Thailand. This analysis includes data from last enrolled patient who received at least 3 months of radotinib therapy. The median age of patients was 47 (range; 24-76) years, and 65 (84.4%) were imatinib-resistant and 12 (15.6%) were imatinib-intolerant. Four patients also had intolerance to dasatinib. With a median follow-up of 10.6 months, treatment with radotinib is ongoing in 46 patients (59.7%) and 31 patients (40.3%) discontinued the treatment including two deaths (2.6%). However, there were no CML-related deaths. Median duration of radotinib exposure was 296 (8-798) days. Overall MCyR rate was 63.6%, including 35 patients (45.4%) complete cytogenetic response and 14 patients (18.2%) partial cytogenetic response. The median time to MCyR was 2.8 months (85 days) and the median duration of MCyR was 315 (range; 5-726) days. Of patients achieving complete cytogenetic response, 37% (13/35) achieved major molecular response. Within follow-up durations, 44 patients (57.1%) required dose interruption and 41 patients (53.3%) had dose reduction. Most common grade 3/4 hematologic and laboratory adverse events (AEs) were thrombocytopenia (27.3%), neutropenia (10.4%), anemia (6.5%), and hyperbilirubinemia (31.2%). Common non-hematologic AEs were rash (29.8%), fatigue (14.3%), nausea/vomiting (14.3%), headache (13.0%), and pruritus (11.7%). The majority of AEs were easily manageable with temporal dose interruption and/or reductions. In all patients with CP-CML treated with second-line radotinib, estimated progression-free survival and overall survival rate at 12 months was 84.9% (95% CI, 72.7-92.0%) and 97.4% (95% CI, 89.9-99.3%), respectively. **Conclusion** Radotinib phase 2 trial confirmed the efficacy and safety of radotinib 400 mg twice daily in patients with CP-CML after failure to TKIs. Most of the AEs occurred in the early treatment period, were tolerable, and were easily controlled by dose interruption or reduction.

[696] Targeting STAT5 Expression Resulted in Molecular Response Improvement in Patients with Chronic Phase CML Treated with Imatinib. Rousselot, Versailles. Background. We have previously demonstrated that PPAR- γ agonists (such as pioglitazone) negatively regulate the level of *Stat5A* and *Stat5B* gene expression in normal CD34+ bone marrow progenitors (Prost, Le Dantec et al. 2008). **Aim.** We investigated whether targeting Stat5 expression with pioglitazone may impact clonogenic activity of Chronic Myelogenous Leukemia (CML) cells *in vitro* and result in molecular response improvement *in vivo*. **Patients and methods.** Preliminary *in vitro* studies tested the ability of pioglitazone to impact viability and clonogenicity of CD34+ primary cells from CML patients (pts). We conducted clonogenic and LTC-IC studies. Cultivated Ph+ -CD34+ cells were characterized by facs and analyzed by CSFE assay. BCR-ABL and Stat5 expression were quantified by real time PCR. Control experiments were conducted using lentiviral vectors and siRNA assay for Stat5 and PPAR-g. CML pts were eligible in the ACTIM trial (EudraCT 2009-011675-79) if they were treated by imatinib for at least 2 years with a stable daily dose for at least 3 months and in major molecular response without having achieved CMR4.5 (defined by a BCR-ABL/ABL IS ratio $\leq 0.0032\%$). After inclusion, pts received imatinib (no dose modification) and started pioglitazone (Actos®) 30 mg/d during 2 months and 45 mg/d thereafter for 12 months. BCR-ABL transcript level was monitored every 3

months during the study period. Primary objective was the proportion of patients achieving a confirmed undetectable level of BCR-ABL transcript. Secondary objectives included cumulative incidence of CMR4.5 and safety. A companion biologic study evaluated imatinib through levels, Stat5 expression in bone marrow at baseline, months 6 and 12 and clonogenic activity of bone marrow mononuclear cells at baseline, months 6 and 12. **Results.** From the *in vitro* studies we first demonstrated that pioglitazone at pharmacological doses inhibited cell growth of the Bcr-Abl positive cell line K562 through the activation of the PPAR-g/STAT5 pathway. We next showed that PPAR-g / STAT5 pathway induced a clonogenic defect in CD34+ cells from CML patients. Moreover, the activation of the PPAR-g / STAT5 pathway also induced a clonogenic and a proliferative defect in CML LTC-IC. We then confirmed that imatinib induced a selection of insensitive quiescent CML cells and showed that this effect was abrogated by the activation of the PPAR-g / STAT5 pathway. Twenty seven pts were enrolled in the clinical trial and 24 were evaluable (1 was excluded in CMR4.5, 1 pt was not in MMR and 1 pt had consent withdrawal). Median age was 61.6 years (24.1-79) and median follow-up after inclusion was 13 months (9.8-21). All evaluable pts started pioglitazone as planned. Seven pts (29.2%) discontinued pioglitazone before 12 months, 6 following investigator decision after the warning of the French ministry of health regarding the risk of bladder cancer and 1 after its own decision. No pt discontinued due to adverse events. Discontinuations occurred between month 3 and month 9. Median duration of pioglitazone therapy was 11.2 months (2.6-15.4) median daily dose was 39.9 mg. No interaction was observed between imatinib and pioglitazone in term of through level before (median 850 ng/ml) and after (median 927 ng/ml) pioglitazone initiation (p=ns). Main adverse events were weight gain and worsening fluid retention in 3 pts. Three pts (14%) obtained a confirmed undetectable level of BCR-ABL transcript. The one year cumulative incidence of CMR4.5 was 57%. Stat5 mRNA quantification was significantly diminished in pt samples at M6 and M12 compared to the baseline values and a reduction of the clonogenic potential was also observed in bone marrow cells at M6 and M12. We collected "control pts" with similar characteristic (n=20). None of these pts obtained a confirmed CMR and the cumulative incidence of CMR4.5 in this control group was 27% as compared to 57% in the pioglitazone group (p=0.02). **Conclusion.** We have extended our *in vitro* results showing that PPAR- γ agonists resulted in Stat5 down regulation in CML CD34+ cells and preferentially reduced their clonogenic and long term potency in CFCs and LTC-IC assays. We now demonstrated that these effects translate *in vivo* by the achievement of MMR in more than half of the pts treated with the combination of pioglitazone and imatinib suggesting that it may be possible to target quiescent CML cells *in vivo* and supporting the concept of stem cell pool erosion.

7 CML Biology and Pathophysiology, excluding Therapy I [907-912]

[907] C/EBP β Is Upregulated Through STAT5 and Accelerates Exhaustion of Leukemic Stem Cells During BCR-ABL-Mediated Myeloid Expansion Yoshihiro Hayashi, Tokyo. Enhanced proliferation and differentiation of myeloid cells are features common to emergency granulopoiesis and chronic phase of chronic myeloid leukemia (CP-CML). We have previously shown that C/EBP β , a transcription factor regulating emergency granulopoiesis, is upregulated in hematopoietic stem/progenitors in CP-CML and that C/EBP β promotes BCR-ABL-mediated myeloid cell expansion (ASH annual meeting abstract. 2011; 118: 3747). However, the molecular mechanisms involved in the upregulation of C/EBP β and the effects of C/EBP β on the CML stem cells remained to be elucidated. Here we show that STAT5 is involved in the BCR-ABL-mediated C/EBP β upregulation and that C/EBP β accelerates the exhaustion of CML stem cells. In order to investigate the regulation of C/EBP β in CP-CML, BCR-ABL gene was retrovirally introduced into EML cells, a mouse hematopoietic stem cell line, to make EML-BCR-ABL. The expression of C/EBP β was upregulated at mRNA and protein level in EML-BCR-ABL when compared to EML cells transduced with a control vector. The upregulation of C/EBP β was significantly repressed by adding imatinib mesylate, suggesting that the downstream signaling pathway of BCR-ABL is directly involved in the process. Neither a PI3K inhibitor nor a MEK inhibitor affected the levels of C/EBP β in EML-BCR-ABL, but a STAT5 inhibitor reversed the upregulation of C/EBP β in EML-BCR-ABL. Retroviral transduction of dominant-negative STAT5 mutant also impaired the upregulation of C/EBP β in EML-BCR-ABL. Transduction of constitutively-active STAT5 mutant into parental EML cells significantly upregulated C/EBP β expression. These results suggest that BCR-ABL upregulates C/EBP β expression at least in part through activation of STAT5. To clarify the role of C/EBP β in the regulation of CML stem cells, BCR-ABL-transduced bone marrow (BM) cells from C/EBP β knockout (KO) mice or wild type (WT) mice were serially transplanted into irradiated recipient mice. In the first round of transplantation, all the recipient mice developed myeloproliferative status and the mice transplanted with BCR-ABL-transduced C/EBP β KO BM cells survived longer than the mice transplanted with BCR-ABL-transduced WT BM cells. Interestingly, the frequency of c-kit⁺ cells within BCR-ABL⁺ cells was higher in the BM of the mice transplanted with BCR-ABL-transduced C/EBP β KO cells than in the BM transplanted with BCR-ABL-transduced WT cells (Figure). When 2×10^6 BCR-ABL⁺ BM cells from primary recipient mice were transplanted into sublethally irradiated secondary recipients, all the mice developed a myeloproliferative status. Transplantation of 1×10^6 BCR-ABL⁺ BM cells from primary recipient mice, four out of the five recipient mice that transplanted with BCR-ABL-transduced C/EBP β KO BM cells developed a myeloproliferative status and only one out of the four recipient mice that transplanted with BCR-ABL-transduced WT BM cells developed a myeloproliferative status. Transplantation of less than 0.5×10^6 BCR-ABL⁺ BM cells engrafted none of the secondary recipient mice. The frequencies of leukemia-initiating cells in BM of primary recipient mice that transplanted with BCR-ABL-transduced WT cells or BCR-ABL-transduced C/EBP β KO cells were 1 in 1,404,129 and 1 in 683,773, respectively. These findings suggest that C/EBP β promotes exhaustion of CML stem cells. In conclusion, these results suggest that C/EBP β is upregulated by BCR-ABL at least in part through STAT5 and that C/EBP β has the ability to exhaust CML stem cells. We are currently investigating the molecular mechanisms which protect CML stem cells from C/EBP β -mediated exhaustion for complete eradication of CML stem cells in the future.

[908] Molecular Characterization of the Leukemic Niche in Chronic Myeloid Leukemia (CML) and Evaluation of a Leukemia / Niche Cross-Talk Aggoune There is growing evidence that the bone marrow microenvironment could participate to the progression of chronic myeloid leukemia (CML). Recent data show indeed that placental growth factor (PGF) expression is highly induced in stromal cells from CML patients although they are not part of the leukemic clone as they are Ph1-negative (Schmidt et al, Cancer Cell 2011). It is possible that leukemic cells instruct the niche components via extracellular or contact signals, transforming progressively the "normal niche" into a functionally "abnormal niche" by

inducing aberrant gene expression in these cells, similar to the pattern that has been identified in cancer-associated fibroblasts (CAF). In an effort to identify the differential gene expression pattern in the CML niche, we have undertaken two strategies of gene expression profiling using a Taqman Low Density Arrays (TLDA) protocol designed for 93 genes involved in antioxidant pathways (GPX, PRDX, SOD families), stromal cell biology (Collagen, clusterin, FGF, DHH), stem cell self-renewal (Bmi1, MITF, Sox2) and hematopoietic malignancies (c-Kit, hTERT, Dicer, beta-catenin, FOXO3). The first strategy consisted in the analysis of mesenchymal stem cells (MSCs) isolated from the bone marrow of newly diagnosed CP-CML patients (n=11). As a control, we have used MSCs isolated from the bone marrow of age-matched donors (n=3). MSCs were isolated by culturing 6-8.10⁶ bone marrow mononuclear cells in the presence of b-FGF (1 ng/ml). At 2-3 weeks, cells were characterized by the expression of cell surface markers (CD105+, CD90+) and by their potential of differentiation towards osteoblastic, chondrocytic and adipocytic lineages. The second strategy aimed to study the potential instructive influence of leukemic cells in the gene expression program of normal MSC after co-culture with either the UT7 cell line expressing BCR-ABL (3 days) or with CD34+ cells isolated from CP-CML at diagnosis (5 days) as compared to co-culture with cord blood CD34+ cells. After culture, CD45-negative MSC were cell-sorted and analyzed by TLDA. All results were analyzed using the StatMiner software. **Results:** TLDA analysis of gene expression pattern of MSC from CML patients (n=11) as compared to normal MSCs (n=3) identified 6 genes significantly over-expressed in CML-MSC: PDPN (10-Fold Increase), V-CAM and MITF (~8 Fold increase), MET, FOXO3 and BMP-1 (~5 Fold increase). To confirm these results we have performed Q-RT-PCR in a cohort of CML-MSC (n= 14, including the 11 patients as analyzed in TLDA) as compared to normal MSC. High levels of PDPN (Podoplanin, ~8 fold increase), MITF (Microphthalmia Associated Transcription factor, 4-Fold) and VCAM (Vascular Cell Adhesion Protein, 2 fold increase) mRNA were again observed on CML MSCs. Our second strategy (co-culture of normal MSC with BCR-ABL-expressing UT7) revealed an increase of IL-8 and TNFR mRNA expression in co-cultured MSCs (~5-fold) whereas there was a major decrease in the expression of DHH (~25-fold) upon contact with BCR-ABL-expressing cells. No modification of the expression of PDPN, MITF or VCAM was noted in normal MSC after this 3-day co-culture strategy using UT7-BCR-ABL cells. Current experiments are underway to determine if primary CD34+ cells from CML patients at diagnosis could induce a specific gene expression pattern in normal MSC after 5 days of co-culture. PDPN is a glycoprotein involved in cell migration and adhesion, acting downstream of SRC. It has been shown to promote tumor formation and progression in solid tumor models and is highly expressed in CAFs. MITF is a bHLH transcription factor involved in the survival of melanocyte stem cells and metastatic melanoma. Finally, high VCAM1 mRNA expression by MSCs from CML patients could be involved in increased angiogenesis known to be present on CML microenvironment. In conclusion, our results demonstrate an abnormal expression pattern of 3 important genes (PDPN, MITF and VCAM1) in MSC isolated in CP-CML patients at diagnosis. The mechanisms leading to an increased mRNA expression (instructive or not instructive by leukemic cells) and their relevance to CML biology are under evaluation. Our results, confirming previous data, suggest strongly the existence of a molecular cross-talk between leukemic cells and the leukemic niche. The elucidation of such aberrant pathways in the microenvironment could lead to the development of "niche-targeted" therapies in CML.

[909] Genomic Instability in CML-CP originates From the Most Primitive Imatinib-Refractory Leukemia Stem Cells

Elisabeth Bolton Philadelphia. Genomic instability is a hallmark of chronic myeloid leukemia in chronic phase (CML-CP) resulting in the appearance of clones carrying BCR-ABL1 kinase mutations encoding resistance to tyrosine kinase inhibitors (TKIs) and/or those harboring additional chromosomal aberrations, eventually leading to disease relapse and/or malignant progression to blast phase (CML-BP) [Skorski, T., Leukemia and Lymphoma, 2011]. We found that Lin⁻CD34⁺CD38⁻ human leukemia stem cells (huLSCs), including the quiescent sub-population, and Lin⁻CD34⁺CD38⁺ human leukemia progenitor cells (huLPCs) accumulate high levels of reactive oxygen species (ROS) resulting in numerous oxidative DNA lesions such as 8-oxoguanine (8-oxoG) and DNA double-strand breaks (DSBs) [Nieborowska-Skorska, Blood, 2012]. huLSCs and huLPCs treated with TKIs continue to exhibit ROS-induced oxidative DNA damage suggesting the persistence of genomic instability in TKI-treated patients. Furthermore, genomic instability in TKI-refractory huLSCs and TKI-sensitive huLPCs may have a varying impact on disease progression and determining novel treatment modalities. To determine if TKI-refractory huLSCs are a source of genomic instability we employed a tetracycline-inducible murine model of CML-CP: SCL1TA/p210BCR-ABL1. Mice exhibiting CML-CP-like disease demonstrated splenomegaly, leukocytosis, and expansion of mature Gr1⁺CD11b⁺ cells. ROS were elevated in Lin⁻c-Kit⁺Sca-1⁺ cells (muLSCs), but not Lin⁻c-Kit⁺Sca-1⁻ cells (muLPCs), which was associated with higher mRNA expression of BCR-ABL1 in muLSCs. In addition, ROS levels were directly proportional to BCR-ABL1 kinase expression in transduced CD34⁺ human hematopoietic cells, thus confirming the "dosage-dependent" effect of BCR-ABL1 on ROS. Among the Lin⁻c-Kit⁺Sca-1⁺ cells, enhanced ROS were detected in TKI-refractory quiescent muLSCs, in CD34⁺Fit3⁻ long-term and CD34⁺Fit3⁺ short-term muLSCs, and also in CD34⁺Fit3⁺ multipotent progenitors. High levels of ROS in muLSCs were accompanied by aberrant expression of genes regulating ROS metabolism (mitochondrial electron transport, oxidative phosphorylation, hydrogen peroxide synthesis, and detoxification). In addition, muLSCs, including the quiescent sub-population, displayed high levels of oxidative DNA lesions (8-oxoG, and DSBs). ROS-induced oxidative DNA damage in muLSCs was accompanied by genomic instability in CML-CP-like mice, which accumulated a broad range of genetic aberrations recapitulating the heterogeneity of sporadic mutations detected in TKI-naive CML-CP patients. These aberrations include TKI-resistant BCR-ABL1 kinase mutations, deletions in *Ikzf1* and *Trp53* and additions in *Zfp423* and *Idh1* genes, which have been associated with CML-CP relapse and progression to CML-BP. Imatinib caused only modest inhibition of ROS and oxidative DNA damage in TKI-refractory muLSCs. In concordance, CML-CP-like mice treated with imatinib continued to accumulate genomic aberrations. Since BCR-ABL1(K1172R) kinase-dead mutant expressed in CD34⁺ human hematopoietic cells did not enhance ROS, it suggests that BCR-ABL1 kinase-independent mechanisms contribute to genomic instability. In summary, we postulate that ROS-induced oxidative DNA damage resulting in genetic instability may originate in the most primitive TKI-refractory huLSCs in TKI-naive and TKI-treated patients.

[910] Combined JAK/STAT5A and BCR-ABL Inhibition Impairs Blast Crisis Chronic Myeloid Leukemia Stem Cell Self-Renewal. Court Recart, La Jolla. In blast crisis transformation of CML (BC CML), the leukemia stem cells (LSC), via the acquisition of both enhanced survival and self-renewal capacity, become increasingly resistant to BCR-ABL targeted

tyrosine kinase inhibition and thus often contribute to relapse after treatment, pointing to the need for alternative therapeutic strategies and a better understanding of the molecular mechanisms underlying disease progression. Janus kinase 2 (JAK2) plays an important role in BCR-ABL⁺ cell survival and has profound effects on self-renewal and lineage commitment of normal and leukemic hematopoietic stem cells, through the activation of the transcription factor signal transducer and activator of transcription 5 (STAT5). To determine if JAK/STAT signaling pathway activation is related to CML progression, LSC from human Chronic Phase (CP CML) and BC CML samples were sorted using FACS Aria (Lin-CD34+CD38+) and analyzed using splice-isoform specific q-RT-PCR. Our results showed that, compared to CP CML, BC LSC harbor enhanced mRNA expression of BCR-ABL, JAK2 and STAT5A isoforms, confirming that progression of CP to BC, in CML LSC, is marked by activation of JAK/STAT pathway. Therefore, we investigated the response of BC CML LSC to a clinical grade JAK2 inhibitor, SAR302503 (Sanofi, Cambridge, MA) alone or in combination with a potent BCR-ABL inhibitor, dasatinib, *in vivo*. After two weeks of treatment, RAG2^{-/-}g_c^{-/-} mice intrahepatic transplanted with BC LSC, showed a significant (p<0.05) reduction of engraftment levels, after combination therapy with SAR302503 and dasatinib, compared to vehicle treated mice, in four different patient samples. In all the hematopoietic tissues analyzed, SAR302503 alone (60 mg/kg/b.i.d.) did not have an effect reducing the leukemic burden. Dasatinib alone (50mg/kg/day) reduced the LSC population in the liver, spleen, and peripheral blood, but the bone marrow retained a significant percentage of BC LSC. However, combination treatment was able to reduce the LSC in the BM significantly (p=0.0006) compared to dasatinib alone. To test whether the combination therapy can impair self-renewal capacity of the BC CML LSC *in vivo*, we immunomagnetic bead selected CD34⁺ cells from BM and spleens of treated mice, and serially transplanted an equal number into secondary recipients. We observed a significant (p<0.0001) reduction of engraftment of LSC on the mice transplanted with combination treated cells compared with vehicle treated cells. Interestingly, secondary mice transplanted with cells treated with dasatinib showed 37.5% engraftment in the spleen and 46.4% in BM, while the level of engraftment for mice transplanted with combination treatment was only 1% and 3% for spleen and BM, respectively. Moreover, mice serially transplanted with combination treated cells, had a significant (p=0.0002) increased survival time. BC CML LSC are enriched for the granulocyte macrophage progenitor (GMP) population, which has been shown to harbor LSC serial transplantation potential. Our results showed that secondary recipient transplanted with combination treated cells, presented a significantly lower proportion of the GMP population, compared with vehicle (p<0.0001) and dasatinib (p=0.02) treated cells. Together, these results suggest that the combination therapy, using a Jak2 inhibitor with a BCR-ABL inhibitor, can abolish LSC self-renewal capacity and thereby potentially prevent relapse. Validation studies, using nanoproteomic analysis, confirmed that LSC sorted cells from mice treated with SAR302503 had lower expression levels of p-JAK2 (Tyr 1007-08) and p-STAT5A (Tyr 694) compared with vehicle treated mice (51% and 64% of reduction, respectively), while no changes are observed for total JAK2 protein or B2M between both conditions. Full transcriptome sequencing and q-RT-PCR analysis, on sorted CML LSC from mice treated with SAR302503 in combination with dasatinib, confirmed that STAT5A specific isoforms decreased after treatment, suggesting JAK/STAT pathway could be used as biomarker of response and could explain the impairment of self-renewal in the combination therapy.

[911] Loss of Krüppel-Like Factor 4 (KLF4) Impairs the Self-Renewal of Leukemic Stem Cells. Park, Texas. The identification of pathways that differentially regulate the self-renewal in normal hematopoietic stem cells (HSC) and leukemic stem cells (LSC) is critical for effective treatment of relapsed leukemia without affecting normal hematopoiesis. Krüppel-like factor 4 (KLF4) is a bi-functional transcription factor that can either activate or repress transcription and thus function as an oncogene or a tumor suppressor depending on the cellular context. We have previously shown that loss of KLF4 leads to increased self-renewal of HSC in serial bone marrow transplantation. In this work, we investigated whether KLF4 also regulates self-renewal in LSCs using a mouse model of somatic deletion of KLF4 in hematopoietic cells (*Klf4^{fl/m} Vav-1Cre⁺*) and the BCR-ABL-induced chronic myeloid leukemia (CML) model. Mice transplanted with wild type bone marrow (BM) cells transduced with retrovirus carrying BCR-ABL (p210) (n=18) showed an expansion of myeloid cells (GFP⁺ Gr1⁺) with a median survival of 19 days. In contrast, mice transplanted with KLF4-deficient BM cells transduced with BCR-ABL retrovirus (n=12) exhibited prolonged survival with a median survival of 27 days (p<0.0011). Progression of leukemia was monitored in peripheral blood, BM and the spleen by flow cytometry. Mice transplanted with KLF4-deficient cells showed expansion of myeloid leukemic cells (GFP⁺ Gr1⁺) in the first two weeks after BM transplant, which was followed by a progressive loss of myeloid cells and an expansion of B cells (GFP⁺ B220⁺). In control group, 90% of leukemic mice succumbed from CML whereas more than 50% of KLF4-deficient leukemic mice developed mixed CML and B-ALL leukemia. These results suggest that loss of KLF4 impairs the maintenance of BCR-ABL-induced CML, while allowing expansion of BCR-ABL positive B cells (GFP⁺ B220⁺). Since KLF4 deletion prevented continuous expansion of myeloid leukemic cells (GFP⁺ Gr1⁺), we postulated that KLF4 may be critical to LSC maintenance. Thus, we analyzed the frequency of LSCs (GFP⁺ Lin⁻c-Kit⁺ Sca-1⁺ and GFP⁺ Lin⁻c-Kit⁺ Sca-1⁺ Flt3⁻) in BM and the spleen. We found that LSCs were significantly reduced in recipients of BCR-ABL-transduced KLF4-deficient BM cells 18 days after transplantation (n=6, p <0.001). These studies demonstrate that KLF4 has different roles in the maintenance of LSCs and normal HSCs and that inactivation of KLF4 provides a therapeutic strategy for eradicating LSCs without damaging normal HSC.

[912] Microenvironmental Protection of CML Stem and Progenitor Cells From Tyrosine Kinase Inhibitors Through N-Cadherin and Wnt Signaling. Zhang Duarte. BCR-ABL tyrosine kinase inhibitors (TKI) do not eliminate leukemia stem cells (LSC) in chronic myeloid leukemia (CML), which remain a potential source of relapse. TKI treatment effectively inhibits BCR-ABL kinase activity in CML LSC, indicating that additional kinase-independent mechanisms contribute to LSC preservation. We investigated the role of signals from the bone marrow (BM) microenvironment in protecting chronic phase (CP) CML stem/progenitor cells from TKI treatment. Culture with human BM mesenchymal stromal cells (MSC), immortalized by ectopic expression of telomerase, significantly inhibited apoptosis in CP CML CD34+CD38- primitive and CD34+CD38+ committed progenitors treated with imatinib (5µM, p<0.01), nilotinib (5µM, p<0.01), or dasatinib (150nM, p<0.05). MSC coculture reduced TKI-mediated apoptosis in both dividing and non-dividing CD34+CD38- cells, defined using CFSE labeling, and increased the percentage of undivided CD34+CD38- cells (p<0.05). MSC coculture also enhanced colony forming ability of CML CD34+CD38- (p<0.05) and CD34+CD38+ (p<0.05) cells treated with TKI. Importantly MSC coculture, with or without imatinib treatment, significantly enhanced engraftment of CML CD34+ cells in immunodeficient

NSG mice, both at 4 weeks (without IM: 27.2±5% human CD45+ cells without stroma, 52.8±8% with stroma, $p<0.001$; with IM: 4.9±2% without stroma, 10.1±2% with stroma, $p<0.05$) and at 10 weeks post-transplant (without IM : 1.48±0.2% human CD45+ cells without stroma, 2.37±0.4% with stroma, $p=0.07$; with IM : 0.74±0.2% without stroma, 1.2±0.3% with stroma, $p<0.05$). CML progenitors cultured in a transwell insert over MSC showed increased apoptosis following TKI exposure compared to cells cultured in direct contact with MSC ($p<0.05$). An increased proportion of CML progenitors adhered to MSC after 4 days of TKI treatment (22±4% without IM, 42±5% with IM, $p<0.05$). Antibody or peptide (NCDH) mediated blockade of the N-Cadherin receptor reduced adhesion of CML progenitors to MSC ($p<0.05$), and increased apoptosis of TKI-treated CML progenitors cocultured with MSC ($p<0.05$), indicating an important role for N-Cadherin in MSC-mediated protection of CML progenitors from TKI. Although N-Cadherin expression measured by flow cytometry, Western blot and Q-RT-PCR was lower in CML compared to normal CD34+CD38- and CD34+CD38+ cells, we observed significantly increased N-Cadherin expression in CML cells remaining after 4 days culture with IM and MSC ($p<0.05$). We observed enhanced β -catenin activity in CML progenitors cocultured with MSC, with and without TKI treatment, as manifested by increased β -catenin protein levels and nuclear localization, enhanced expression of β -catenin target genes, and increased TCF/LEF reporter activity. β -catenin levels and target gene expression were increased primarily in MSC-adherent CML progenitors compared to non-adherent cells, and were significantly reduced by antibody or peptide-mediated inhibition of N-Cadherin-mediated adhesion. Using the Duolink labeling technology, we demonstrated increased protein-protein interactions between N-cadherin and β -catenin in CML progenitors cocultured with MSC, with or without TKI treatment. Finally, we showed that addition of exogenous Wnt1 protected CML progenitors from TKI treatment, whereas inhibition of Wnt receptors by DKK1, or inhibition of β -catenin signaling by the small molecule inhibitor ICG001, enhanced apoptosis of CML progenitors cocultured with MSC, suggesting an important role for exogenous Wnt signaling in MSC-mediated protection of CML progenitors from TKI-induced apoptosis. Microarray analysis of gene expression confirmed enrichment of Wnt/ β -catenin and Cadherin related gene sets in CML CD34+ cells cultured on MSC with or without IM, as well as enrichment of genes related to hematopoietic stem cell (HSC) self-renewal, HSC quiescence, and cytokine signaling. In conclusion, our studies reveal an important role for exogenous Wnt- β -catenin signaling, and a close interplay between N-Cadherin and β -catenin, in microenvironment-mediated resistance of CML stem and progenitor cells to TKI treatment. These findings support further development of novel treatment strategies to eradicate residual LSC in TKI-treated CML patients through inhibition of Wnt secretion or blockade of Wnt or N-Cadherin receptors.

8 CML Biology and Pathophysiology, excluding Therapy II [31-36]

[31] STAT3 Inhibition Synergizes with BCR-ABL1 Inhibition to Overcome Kinase-Independent TKI Resistance in Chronic Myeloid Leukemia (CML). Eiring, Salt Lake City. In CML, TKI resistance in the absence of BCR-ABL1 kinase mutations is mechanistically unclear. Since extrinsic signals from the bone marrow (BM) microenvironment protect CML cells from TKIs in a STAT3-dependent manner (Bewry et al. 2008; Traer et al. 2011), we hypothesized that overt resistance may occur when CML cells maintain *intrinsic* STAT3 activation in the absence of *extrinsic* signals. We also asked whether combined targeting of STAT3 and BCR-ABL1 could produce synthetic lethality to overcome TKI resistance. To model *extrinsic* BCR-ABL1 kinase-independent resistance, we grew CML cell lines and CML^{CD34+} progenitor cells from newly diagnosed patients in the presence of conditioned medium (CM) derived from HS-5 BM stromal cells. To model *intrinsic* resistance, we used the imatinib-resistant K562^R and AR230^R cell lines. Our models of TKI resistance proliferate in 1.0-2.5 μ M imatinib and exhibit pSTAT3^{Y705} activation despite suppression of BCR-ABL1 kinase activity. To investigate the role of pSTAT3^{Y705} in TKI resistance, we used shRNA-mediated knockdown (shSTAT3), a dominant-negative mutant (dnSTAT3), and pharmacologic STAT3 inhibitors developed in our laboratories to block STAT3 activity. Compared to scrambled controls in the presence of HS-5 CM, shSTAT3 reduced colony formation (33.8% reduction, $p<0.008$) and increased apoptosis (17.9% increase, $p<0.03$) of parental K562 cells following imatinib exposure, thereby abolishing the protective effect of *extrinsic* BM-derived factors. In contrast, shSTAT3 had no effect on cells grown in regular medium. In *intrinsic* resistance, shSTAT3 reduced clonogenicity of K562^R cells by 58.3% ($p<0.0004$) and AR230^R cells by 62.2% ($p<0.002$) in the presence of 1.0 μ M imatinib, with no effect on TKI-sensitive controls. A dnSTAT3 mutant lacking the C-terminal activation domain also impaired the clonogenicity of K562^R and AR230^R cells by 36.1% ($p<0.003$) and 50.0% ($p<0.004$), respectively. Thus, STAT3 plays a functional role in mediating *intrinsic* and *extrinsic* TKI resistance in BCR-ABL1-expressing cell lines. Preliminary immunofluorescence data indicates that pSTAT3^{Y705} levels are higher in drug-resistant CML CD34⁺CD38⁻ stem cells than in CD34⁺CD38⁺ progenitor cells, suggesting a potential role for pSTAT3^{Y705} in TKI resistance of primitive CML stem cells. To investigate whether pharmacologic STAT3 inhibition in combination with TKIs could overcome resistance, we tested a library of compounds that interfere with STAT3 dimerization. The most selective of these inhibitors, S31-201.1066 (SF1-066), was biochemically shown to disrupt STAT3 dimerization and transcriptional activation. Compared to cells treated with 1.0 μ M imatinib alone, addition of 10 μ M SF1-066 impaired the clonogenicity of K562^R cells by 68.6% ($p<0.03$) and AR230^R cells by 72.5% ($p<0.0004$), with no effect on TKI-sensitive controls. When CML^{CD34+} progenitor cells from newly diagnosed patients ($n=4$) were cultured in the presence of HS-5 CM and treated with the SF1-066 / imatinib combination, clonogenicity following drug exposure was reduced by 45.2% ($p<0.002$) compared to imatinib. Therefore, pharmacologic inhibition of STAT3 and BCR-ABL1 overcomes *intrinsic* and *extrinsic* TKI resistance in both CML cell lines and CML^{CD34+} progenitor cells. We next developed a series of 14 second-generation STAT3 inhibitors and tested them using a luciferase reporter harboring sequential STAT3 cis-inducible elements to measure endogenous STAT3 activity in AR230^R cells. Two lead compounds, BP2-047 and BP3-163, reduced luciferase activity by 69.2% ($p<3.6\times 10^{-6}$) and 51.8% ($p<8.6\times 10^{-6}$), respectively, with little effect on cells expressing a scrambled reporter. Selected inhibitors then provided scaffolds for the synthesis of focused libraries with enhanced potency and selectivity. Immunofluorescence data revealed that two of these inhibitors, BP5-087 and BP5-088, resulted in cytoplasmic accumulation of pSTAT3^{Y705} in CML^{CD34+} progenitors grown in HS-5 CM, consistent with a block of dimerization. Screening and validation of third-generation STAT3 inhibitors are underway and will be reported. Altogether, our data identifies STAT3 as a universal target in TKI-resistant CML cells, and suggests that simultaneous pharmacologic inhibition of STAT3 and BCR-ABL1 may be a suitable therapeutic strategy for treatment of patients with TKI resistance despite inhibition of BCR-ABL1 kinase activity.

[32] Effective Elimination of CML Progenitor and Stem Cells Through Combination of α -CD123 Antibody-Dependent Cell-Mediated Cytotoxicity and Tyrosine Kinase Inhibitor Treatment. Nievergall, Adelaide. Since the introduction of tyrosine kinase inhibitor (TKI) therapy overall survival and complete molecular response rates in chronic phase chronic myeloid leukemia (CP-CML) patients have significantly improved. However, leukemic stem cells (LSCs) and progenitor cells persist and are thought to be responsible for disease progression, development of TKI resistance and disease recurrence after stopping TKI therapy. Protection by cytokines, such as IL-3 and GM-CSF, provides a potential mechanism of LSC resistance. While in acute myeloid leukemia (AML) monoclonal antibody (mAb) targeting of IL-3 receptor α (CD123), a recognized marker for AML LSCs, has been studied *in vitro* and *in vivo*, similar investigations have not been undertaken in CML to date. CSL362 is a genetically-engineered form of the specific blocking mAb 7G3 optimized for Fc receptor binding to achieve maximal antibody-dependent cell-mediated cytotoxicity (ADCC) capacity. Here we investigate the expression of CD123 in CD34⁺ progenitors and CD34⁺CD38⁻ LSCs, isolated from CP- and blast crisis (BC) - CML patients, and study the benefits of targeting those cells by CSL362 alone and in combination with TKIs. Flow cytometry analysis established significantly elevated expression of CD123 on CD34⁺CD38⁻ cells from CP-CML (53.0 \pm 5.8 %, n=16, p=0.003) and BC-CML (73.2 \pm 6.7 %, n=18, p<0.001) patients compared to normal donors (20.3 \pm 4.2 %, n=8), with clear increases in CD123 expression with disease progression in matched samples (n=2). Subsequent assessment of apoptosis, colony forming unit (CFU-GM) and long-term culture-initiating cell (LTC-IC) potential confirmed the ability of CSL362 to block IL-3-mediated rescue of TKI-induced cell death. However, in the presence of other cytokines, likely found in the physiological bone marrow microenvironment, this effect was lost. We also demonstrate by lactate dehydrogenase release and clonogenic assays that CML CD34⁺ cell numbers are significantly reduced, in a dose-dependent manner, by CSL362-induced ADCC employing NK cells from healthy donors (42.4 \pm 8.1 % lysis, n=3, and CFU-GM decreased to 30 \pm 10.8 % of controls, n=5, p=0.003). In keeping with this, flow cytometry analysis revealed specific elimination of CP- and BC-CML CD123⁺ CD34⁺CD38⁻ cells (from 42.9 % to 18.6 %, n=5, p=0.004, and from 71 % to 35.3 %, n=3, p=0.044, respectively). Importantly, autologous CML patient NK cells, collected after achievement of major molecular response, also mediate CSL362-dependent cytotoxicity similar to allogeneic healthy donor NK cells as indicated by equivalent numbers of remaining CFUs (28 \pm 6.7 % vs. 34.9 \pm 3.4 %, n=5, Fig. A). We further have evidence to suggest preferential elimination of CML over normal LTC-ICs (30.3 \pm 9.9 % vs. 62.6 \pm 11.2 % remaining, n=3, p=0.096) in the autologous setting. Of clinical importance, the combination of Nilotinib and CSL362 resulted in a significantly greater reduction in CFUs (additive effect) when compared to either agent alone (Fig. B). Taken together these data suggest that selective ADCC-mediated lysis, likely the major mode of action of CSL362 *in vivo*, efficiently eliminates CML progenitor and stem cells. Promising results evaluating CSL362/TKI combination treatments, with the expectation to further enhance specificity for leukemic while sparing normal progenitor and stem cells as indicated from preliminary experiments, warrant further studies.

A: Autologous NK cells are able to confer CSL362-induced ADCC against CML CD34⁺ cells. Cells were co-cultured at an effector to target cell ratio (E:T) of 10:1 in the absence and presence of CSL362 as indicated for 4 h and remaining CFU-GM were enumerated. Data is normalized to target cells alone (***) p<0.001). **B:** CSL362-mediated ADCC and TKI treatment show additive effects. CP-CML CD34⁺ cells were cultured with nilotinib at varying concentrations as indicated for 48 h before overnight exposure to CSL362 (1 μ g/ml) with or without allogeneic NK cells (E:T 1:1). Mean \pm SE of CFU-GM colony numbers is shown (n=3, * p<0.05, ** p<0.01).

[33] Stress Response Gene Egr-1 As Tumor Suppressor in BCR-ABL Mediated Leukemia. Maifrede, Philadelphia. Chronic Myelogenous Leukemia (CML) is a disease resulting from the neoplastic transformation of hematopoietic stem cells (HSC) with the BCR-ABL oncogene. The BCR-ABL protein is a constitutively active tyrosine kinase, which promotes cell survival and proliferation by means of diverse intracellular signaling pathways, thereby being the culprit for malignant transformation. In the late 1990s a Tyrosine Kinase Inhibitor (TKI), imatinib mesylate (Gleevec, Novartis) started to be effectively used on CML patients. However, imatinib, therapy suppresses rather than eliminates the disease, and resistance to imatinib has been described. Thus there is a high priority to enhance our understanding of how BCR/ABL subverts normal hematopoiesis and to identify novel targets for therapy. The transcription factor early growth response 1 (Egr-1) was identified as a macrophage differentiation primary response gene, shown to be essential for and to restrict differentiation along the macrophage lineage. There's evidence consistent with Egr-1 behaving as a tumor suppressor of leukemia, both *in vivo* and *in vitro*, including (1) loss of Egr-1 associated with treatment derived AMLs; (2) deregulated Egr-1 overriding blocks in myeloid differentiation, and (3) haplo-insufficiency of Egr-1 in mice leading to increased development of myeloid disorders following treatment with the potent DNA alkylating agent, N-ethyl-nitrosourea (ENU). Therefore, we chose to investigate if Egr-1 can act as a suppressor of BCR-ABL driven leukemia. To assess the effect of Egr-1 on BCR-ABL driven leukemia, lethally irradiated syngeneic wild type mice were reconstituted with bone marrow (BM) from either wild type or Egr-1 null mice transduced with a 210-kD BCR-ABL-expressing MSCV-retrovirus (bone marrow transplantation {BMT}). It was observed that loss of Egr-1 accelerated the development of BCR-ABL driven leukemia in recipient mice. Furthermore, we investigated the stem cell compartment of both Egr-1 WT and Egr-1^{-/-} BM, by determining the percentage of stem cells (Lin⁻ Sca⁺c-Kit⁺, LSK), before infection with BCR-ABL; no statistically significant difference in the percentage of LSK cells was observed between Egr-1 WT and Egr-1^{-/-} BM. Thus, the BM stem cell compartment of the Egr-1^{-/-} mice does not offer a quantitative advantage to justify the faster development of leukemia compared to Egr-1 WT mice. Furthermore, when BM of transplanted mice was analyzed we observed an increased population of lineage negative cells in Egr-1^{-/-} BCR-ABL recipients when compared to animals transplanted with WT BCR-ABL BM, consistent with more rapid development of disease. Preliminary results from serial BMT has shown that Egr-1^{-/-} BCR-ABL BM has an increased leukemic burden when compared to the WT counterpart. Additional data from our animal model, as well as analysis of human leukemia samples will be presented, further corroborating that Egr-1 functions as a suppressor of BCR-ABL driven CML. These data could result in novel targets for diagnosis, prognosis, and targeted therapeutics for CML, as well as for other leukemic diseases.

[34] Lineage-Specific Functions of LKB1 in CML and B Lymphoid Blast Crisis. Chan, San Francisco. Background:

The serine-threonine liver kinase B1 (LKB1, also called STK11) acts as negative regulator of aerobic glycolysis, a metabolic pathway that is typically used in cancer cells (commonly referred to as 'Warburg effect'). LKB1, together with AMPKs, shifts the metabolic balance from aerobic glycolysis to oxidative phosphorylation and thereby reverses the metabolic program of cancer cells and functions as tumor suppressor. Recently, it has been shown that LKB1 plays a critical role in the maintenance of quiescence and metabolic homeostasis of hematopoietic stem cells (HSCs). **Hypothesis:** In the present study, we focused on the roles of LKB1 in BCR-ABL1-driven leukemias including CML and B lymphoid blast crisis/Ph⁺ ALL (LBC). While LKB1 is widely seen as tumor suppressor in solid tumors, we found that high expression levels of LKB1 at diagnosis predict poor clinical outcome in patients with high risk acute lymphoblastic leukemia (n=207; COG P9906 trial; p=0.0204). In addition, high levels of LKB1 expression correlate with positive minimal residual disease (MRD+, p=0.0323) status in patients. These findings were unexpected and seem to contradict the common notion of LKB1 as a tumor suppressor. **Results:** To study the function of LKB1 in CML and B lymphoid blast crisis/Ph⁺ ALL (LBC), we developed a mouse model for inducible ablation of *Lkb1* in BCR-ABL1-transformed hematopoietic stem and progenitor cells (CML-like) and B cell progenitors (LBC). To this end, *Lkb1*-fl/fl bone marrow hematopoietic stem and progenitor cells and B cell precursor cells were transformed with BCR-ABL1 and transduced with tamoxifen-inducible Cre. Unexpectedly, Cre-mediated deletion of *Lkb1* had opposite effects in CML and LBC. While *Lkb1*-deletion in CML results in an initial proliferative burst of the leukemia cells, the vast majority of B cell lineage LBC cells undergo rapid cell cycle arrest. These findings are consistent with changes of cell cycle checkpoint proteins in response to *Lkb1* deletion in CML and B cell lineage LBC. While *Lkb1* deletion in CML cells results in downregulation of Arf, p53 and p27 levels, *Lkb1* deletion in B lineage LBC cells resulted in upregulation of Arf and p27. In addition, *Lkb1* deletion in CML resulted in inactivation of AMPK, a known substrate of LKB1, as well as enhanced activation of mTORC1. By contrast, while deletion of *Lkb1* in B cell lineage LBC cells resulted in inactivation of AMPK as shown by reduced phosphorylation of AMPK α T172, there was reduction in mTORC1 activity based on diminished levels of phospho-p70 S6 kinase and S6 following LKB1 deletion. The effects of LKB1 on sensitivity of BCR-ABL1 CML and B lineage LBC cells to Imatinib were also examined. *Lkb1*-deficient ALL cells became more sensitive to Imatinib treatment. On the other hand, initial *Lkb1* deletion rendered CML cells more resistant to Imatinib treatment. When primary patient-derived Ph⁺ ALL cells (n = 3) were treated with Imatinib, upregulation of phospho-LKB1 (S428) was observed. Finally, LKB1 was also shown to regulate energy homeostasis in CML and B cell lineage LBC in different manners, as measured by monitoring ATP and lactate production. **Conclusions:** Here we show that *Lkb1* plays critical roles in mediating proliferation and cell growth in BCR-ABL1-driven leukemias. While LKB1 is widely seen as a tumor suppressor that limits aerobic glycolysis in cancer cells according to the Warburg effect, our findings demonstrate that LKB1 has lineage-specific functions in BCR-ABL1 driven leukemias. While LKB1 function in CML resembles its tumor suppressor function in solid tumors, LKB1 is critical for survival and proliferation on B cell lineage CML blast crisis and Ph⁺ ALL. The finding of a divergent role of *Lkb1* in CML and B cell lineage LBC/Ph⁺ ALL is relevant because small molecule inhibitors of AMPK and mTORC1 are currently under development for the treatment of BCR-ABL1-driven leukemias.

[35] Anti-Leukemic Activity of the CRM1 Inhibitor KPT-330 in Advanced CML and Ph⁺ ALL. Walker, Columbus.

As tyrosine kinase inhibitors (TKIs) do not induce long-term response in blast crisis chronic myeloid leukemia (CML-BC) or Philadelphia chromosome-positive acute lymphoblastic leukemia (Ph⁺ ALL), and are unable to kill quiescent Ph⁺ hematopoietic stem cells, alternative therapies targeting dysregulated pathways in BCR-ABL1⁺ acute leukemias are needed. CRM1, a karyopherin aberrantly overexpressed in several cancers, controls the nuclear export of proteins (e.g. ABL1, SET, p53, p21, FOXO and RB) that regulate normal and malignant hematopoietic cell survival, self-renewal and proliferation. Here we show that enhanced CRM1 expression also occurs in Ph⁺ leukemias and the clinical stage small molecule inhibitor of CRM1, KPT-330, has a detrimental effect on malignant but not normal hematopoietic progenitors. Specifically, a substantial upregulation ($\geq 65\%$ increase) in the expression levels of CRM1 was detected by immunoblot in CD34⁺ progenitors from bone marrow (BM) of leukemic primary samples (n=3), when compared to CD34⁺ progenitors from healthy (n=3) donors. Interestingly, CRM1 protein levels in CML CD34⁺ cells were markedly but not totally reduced by treatment with the ABL1 kinase inhibitor Imatinib (1 μ M, 72h), suggesting that BCR-ABL1-driven pathways are not the only factor contributing to CRM1 upregulation. Additionally, treatment of primary CML cells with KPT-330 (1 μ M, 72h) resulted in a 75% reduction in BCR-ABL1 kinase activity, consistent with an interrelationship between BCR-ABL1 and CRM1 activities. Finally, the ability of BCR-ABL1 to induce CRM1 expression was demonstrated upon ectopic BCR-ABL1 expression in myeloid 32Dcl3 precursor cells in which CRM1 protein levels became ~10-fold higher. To determine the therapeutic relevance of CRM1 inhibition, cell survival was assessed in MACS-purified CD34⁺ progenitors. Treatment with KPT-330 resulted in $\geq 80\%$ induction of apoptosis in Ph⁺ (n=5) CML (2 μ M, 72hr) and B-ALL (500nM, 72h) CD34⁺ cells. Conversely, KPT-330 exerted only a minimal effect on the survival of CD34⁺ progenitors from healthy donor BM (15-30% Annexin V positive, KPT-330 0.5-2 μ M, 72h). Consistent with the existence of BCR-ABL1-independent signaling leading to increased CRM1 expression, marked apoptosis was also observed in KPT-330-treated CD34⁺ progenitors isolated from a Ph-negative B-ALL patient. Thus, CRM1 inhibitor-based therapies might also benefit BCR-ABL1-negative leukemias. To formally determine the effects of KPT-330 on leukemic cell survival, methylcellulose-based clonogenic assays were performed. KPT-330 treatment (1 μ M) resulted in almost total suppression (97% reduction) of the clonogenic potential of leukemic but not normal (30% reduction) CD34⁺ progenitors. Because of evidence that CRM1 directly interacts with the protein phosphatase 2A (PP2A) inhibitor SET, and that CRM1 inhibition alters trafficking of hnRNP A1, a direct regulator of the SET-PP2A interplay in Ph⁺ leukemias, it is conceivable that the anti-leukemic activity of KPT-330 is, at least in part, mediated by PP2A activation. Indeed, KPT-330 treatment (1 μ M, 48hr) of BCR-ABL1⁺ cell lines resulted in full restoration of PP2A activity, comparable to levels observed in BCR-ABL1-negative cells. Accordingly, KPT-330 treatment (1 μ M, 12h) of 32Dcl3^{BCR-ABL1} cells caused a nuclear accumulation (4-fold increase) and cytoplasmic decrease (95% lower) of SET protein levels, as indicated by both confocal microscopy and immunoblots of subcellular fractions. Moreover, KPT-330-treated cells showed altered hnRNP A1 cellular distribution and overall downregulation. However, unexpectedly, hnRNP A1 protein levels were proportionally higher in the cytoplasm and lower in the nucleus, suggesting that the deleterious effect of CRM1 inhibition on hnRNP A1 might not depend on direct inhibition of hnRNP-A1 nuclear export. Because KPT-330 displays favorable ADME properties and has been shown to alleviate leukemic burden using *in vivo* models of different cancers, we are currently testing the anti-leukemic

activity of this compound in a mouse model of CML-BC. We do expect a profound inhibition of leukemogenesis, and all treated mice are currently alive with no signs of toxicity. Thus, CRM1 inhibition by KPT-330 represents a potential new therapeutic avenue which could easily be exploited in malignancies like CML-BC and Ph⁺ ALL that show a dismal outcome to current available therapies.

[36] BCR-ABL-Induced Transcriptional Repression of the Interferon Regulatory Factor 8 (IRF-8/ICSBP) Leads to Depletion of Plasmacytoid Dendritic Cells (PDC), Which May Contribute to Leukemogenesis in a Murine Model of Chronic Myeloid Leukemia. Inselmann, Marburg. Introduction: Chronic myeloid leukemia (CML) is caused by the BCR-ABL oncogene. CML patients lack expression of IRF-8 - an interferon-regulated transcription factor that has been shown to exert tumor suppressor functions. IRF-8 is also critical for the development of a rare dendritic cell population, so called plasmacytoid dendritic cells (PDC). PDC are quantitatively significantly reduced or absent in the peripheral blood of first diagnosis CML patients. PDC are also the major producers of IFN-alpha (IFN α) in man. IFN α is a cytokine that has significant therapeutic efficiency in the treatment of CML patients. We here wished to experimentally test, whether BCR-ABL expression and loss of IRF-8 may be causally linked to a reduction of PDC in murine CML and whether there could be any functional relevance for PDC loss in CML development or treatment. **Methods:** PDC counts were studied from peripheral blood samples of primary CML patients at diagnosis, at the time of remission or from healthy donors. PDC function was assessed in vitro by treatment of magnetic bead-enriched PDC with Toll-like receptor 9-specific oligos (ODN 2216) and subsequent assessment of the intracellular IFN α expression in stimulated PDC. A supposed link between BCR-ABL expression, IRF-8 repression and loss of PDC counts was studied in vivo using a murine CML transduction-transplantation model (C57/Bl6 mice, 7Gy sub-lethal irradiation for conditioning). Multiparameter flow cytometry and cell sorting were performed to analyze and enrich, BCR-ABL-positive (GFP⁺) hematopoietic subpopulations and PDC in order to then quantitate their IRF-8 and BCR-ABL transcript level by RT-PCR. In order to also test the functional relevance of PDC during CML leukemogenesis, CML mice were injected intravenously, weekly from day +5 after transplantation with in vitro generated PDC. Mice were simultaneously also s.c.-injected weekly with ODN 2216 to stimulate IFN α secretion in adoptively transferred PDC in vivo. **Results:** As previously reported, newly diagnosed CML patients displayed a significantly reduced PDC count when compared to healthy donors (p<0.001). Upon remission induction with imatinib, PDC counts restored partially, but to a much lesser extend in patients successfully treated with IFN α therapy. Importantly, albeit significantly reduced in number, BCR-ABL-positive first diagnosis CML PDC seem to be functionally intact: CML and healthy donor PDC produced comparable amounts of IFN α in response to Toll-like receptor 9 -specific CpG ODN 2216 stimulation. This suggested that BCR-ABL may compromise PDC function by quantitative rather than qualitative dysregulation. CML mice developed a fatal, BCR-ABL-positive myeloproliferation within 13 to 29 days with 88% penetrance. Compared to control mice (n=8), CML mice (n=14) showed a 7-fold and 3-fold reduction of the frequency of B220⁺mPDCA-1⁺ PDC in bone marrow and spleen, respectively. This was associated with a statistically significant (4-fold) suppression of IRF8 mRNA expression in sorted BCR-ABL(GFP)-positive PDC relative to BCR-ABL-negative PDC from the same mice (n=3) or from control transplantations (n=5). By RT-PCR, there was a trend also for lower IRF8 expression in CML progenitor cells (Lin⁻ c-Kit⁺ Sca-1⁻ GFP⁺), but not in the stem cell enriching population (Lin⁻ c-Kit⁺ Sca-1⁺ GFP⁺). This implied that IRF8 expression is lost during BCR-ABL-induced leukemogenesis in more mature compartments, which supposedly include PDC precursors. Intriguingly, a once weekly adoptive transfer of in vitro generated (to > 30% enriched) PDC for three successive weeks combined with a once weekly subcutaneous injection of CpG ODN 2216 for three weeks was sufficient to almost double survival of CML mice. **Conclusions:** Using a murine model of CML, we provide first experimental evidence that BCR-ABL induced myeloproliferation is causally linked to a quantitative suppression of PDC, and that this is associated with a BCR-ABL-mediated suppression of IRF8 transcription. Since adoptively transferred PDC were capable of counteracting murine CML development, BCR-ABL may facilitate leukemogenesis in part by obstructing PDC maturation. PDC could thus be a novel immunological effector cell population that exerts and/or integrates anti-leukemic immune responses in CML.

9 Biology and Pathophysiology, excluding Therapy: Poster I

1662 Identification of a Unique Autophagy Gene Expression Signature in CD34⁺ CML Stem/Progenitor Cells That Correlates with Clinical Response to Imatinib Mesylate

1663 BMP Pathway Alterations in Chronic Myelogenous Leukemia Lead to the Amplification of Primitive Progenitors Output in Chronic Phase

1664 Inhibition of Autophagy in Combination with Ponatinib or Dual PI3K/mTOR Inhibition to Improve Treatment Response for Both Bcr-Abl Dependent and Independent Mechanisms of TKI-Resistance in CML

1666 *Nfk1* Plays a Tumor-Suppressing Role in BCR-ABL-Induced Leukemias

1667 Chaetocin Exhibits Anti-Leukemia Activity Against Chronic Myeloid Leukemia Stem Cells

1668 Apoptosis in Chronic Myeloid Leukemia Cells Transiently Treated with Imatinib or Dasatinib Is Caused by Residual BCR-ABL Kinase Inhibition

1669 CML Patients Show Sperm Alterations At Diagnosis That Are Not Improved On Tyrosine Kinase Inhibitor Treatment

1670 Combining Dose-Response Slope with in Vitro Sensitivity to ABL Tyrosine Kinase Inhibitors As an Improved Correlative Metric for Predicting Clinical Response in Chronic Myeloid Leukemia

1671 Low Level BCR-ABL Mutations Below the Detection Limit of Current Standard Screening Techniques Occur Predominantly in the CD34⁺ Progenitor Cell Compartment in Chronic Phase CML Patients At Diagnosis. A Substudy of the ENEST1st Trial

1672 PRKD2 Serine-Threonine Kinase, an Essential Effector of Gabp Transcription Factor, Is Required for Development of Chronic Myelogenous Leukemia

1673 BCR-ABL Transformation Requires Glycogen Synthase 1 (GYS1) Expression for Cell Growth and Increased Glycogen Production

1674 Chronic Myeloid Leukemia with Variant t(9;22) Shows Dysregulated Expression of Genes Included in Pivotal Cellular Pathways

10 Biology and Pathophysiology, excluding Therapy: Poster II

2769 Macrocytosis and Iron Stores in Patients with Chronic Myeloid Leukemia Being Treated with Tyrosine Kinase Inhibitors

2770 Longterm Serial Transplantability of BCR-ABL+ Chronic Phase CML Cells Is Predicted by a 6-Week LTC-IC Assay

2771 Three-Dimensional Nuclear Telomeric Organization (3D) of Chronic Myeloid Leukemia Patients Predicts Accelerated Phase and Blast Crisis

2772 The RNA Editase ADAR1 Promotes Malignant Progenitor Reprogramming in Chronic Myeloid Leukemia
 2773 Analysis of Dasatinib-Induced Molecular Mechanisms of Apoptosis in Hypoxia-Adopted CML Cells Utilizing Quantitative Proteomics Technology
 2774 A Novel Emodin Derivative Inhibited Imatinib-Resistant Chronic Myelogenous Leukemia Cells
 2775 CML Patients with Philadelphia-Negative Clones Occurring During Treatment with Tyrosine Kinase Inhibitors Harbor Molecular Mutations Typical for MDS
 2776 Metastasis Suppressor 1 Is Downregulated in CML Stem Cells and Overexpression Impairs Early Leukemic Cell Propagation
 2777 *BCR/ABL* Transcript At 3 Months Predicts Long-Term Outcomes Following Second Generation Tyrosine Kinase Inhibitor Therapy in the Patients with CML in Chronic Phase Who Failed Imatinib
 2778 Suppression of CML Progenitor but Not Stem Cells Requires Simultaneous Inhibition of KIT and BCR-ABL1
 2779 Myeloid-Derived Suppressor Cells Increase in Chronic Myeloid Leukemia and Exert Immune Suppressive Activity
 2780 The Cytoskeleton Proteins VASP and Zyxin Participate in Hematopoiesis and in the BCR-ABL Signaling Pathway

11 Biology and Pathophysiology, excluding Therapy: Poster III

3726 Evaluation of Leukemic Stem Cell Persistence in Chronic Myeloid Leukemia (CML) Patients in Complete Molecular Remission Induced by First Line TKI Therapies
 3727 Integrated Analysis of Whole-Exome Sequencing and Micromas Expression in Blast Crisis Transformation of Chronic Myeloid Leukemia
 3728 A Single Chronic Myeloid Leukemia (CML) Stem Cell Harboring Both BCR-ABL and JAK2-V617F Mutation Modeled by Induced Pluripotency
 3729 Chronic Myeloid Leukemia Patients Undergoing Interferon Alpha Therapy Exhibit Normal Peripheral Blood Gamma Delta T Cells That May Be Expanded *in Vitro* to Generate Predominantly CD45RA-Positive Effector Memory Cells for Immunotherapy
 3730 Global DNA Methylation Analysis Identifies Key Pathway Differences Between Poor (Low OCT-1 Activity) and Standard Risk CP-CML Patients At Diagnosis
 3731 BCR-ABL-Induced Downregulation of *GADD45G* in Chronic Myeloid Leukemia
 3732 Design and Application of a Novel PNA Probe for the Detection At a Single Cell Level of BCR-ABL T315I Mutation in Chronic Myeloid Leukemia Patients
 3733 Proteomic Signature of CD34+ Cells From Chronic Myeloid Leukemia Patients
 3734 Cbl Controls the Expression of Axl and Lyn Tyrosine Kinases Mediating Resistance to Nilotinib in Chronic Myeloid Leukemia Cells
 3735 Combination of Imatinib with CXCR4 Antagonist BKT140 Overcomes the Protective Effect of Stroma and Targets CML *In Vitro* and *In Vivo*
 3736 Commitment of CML Cells to Apoptotic Cell Death Depends On the Length of Exposure to Das and the Level of STAT5 Activity
 3737 Combining ABL1 Kinase Inhibitor, Imatinib and the Jak Kinase Inhibitor TG101348: A Potential Treatment for Residual BCR-ABL Positive Leukemia Cells
 3738 Hepatitis B Virus Reactivation in Chronic Myeloid Leukemia Treated with Various Tyrosine Kinase Inhibitors: Multicenter, Retrospective Study
 3739 Sabutoclastax, a Novel Pan BCL2 Family Inhibitor, Sensitizes Dormant Blast Crisis Chronic Myeloid Leukemia Stem Cells to Dasatinib

12 Therapy: Poster I

1675 Early Response (Molecular and Cytogenetic) and Long-Term Outcomes in Newly Diagnosed Chronic Myeloid Leukemia in Chronic Phase (CML-CP): Exploratory Analysis of DASISION 3-Year Data
 1676 Enestnd 4-Year (y) Update: Continued Superiority of Nilotinib Vs Imatinib in Patients (pts) with Newly Diagnosed Philadelphia Chromosome-Positive (Ph+) Chronic Myeloid Leukemia in Chronic Phase (CML-CP)
 1677 Dynamics and Characteristics of BCR-ABL1 Multiple Mutations in Tyrosine Kinase Inhibitor Resistant CML
 1678 The BCR-ABL Transcript Levels At 3 and 6 Months Predict the Long-Term Outcome of Chronic Myeloid Leukemia Patients Treated Frontline with Imatinib Mesylate: A Gimema CML WP Analysis
 1679 Progressive Peripheral Arterial Occlusive Disease and Other Vascular Events During Nilotinib Therapy in Chronic Myeloid Leukemia
 1680 The BCR-ABL1 Transcript Type Does Not Influence the Response and the Outcome of Chronic Myeloid Leukemia Patients Treated Frontline with Nilotinib
 1681 EUTOS Score Is Predictive of Event-Free Survival, but Not for Progression-Free and Overall Survival in Patients with Early Chronic Phase Chronic Myeloid Leukemia Treated with Imatinib: A Single Institution Experience
 1682 Dasatinib Therapy Affects Bone Homeostasis in Patients with Chronic Myelogenous Leukemia in Chronic Phase Independently of Molecular Response
 1683 A Single Nucleotide Polymorphism in the Coding Sequence of BIM Is Associated with Poor Prognostic in Chronic Myeloid Leukaemia Treated by Imatinib
 1684 Sustained Molecular Response with Maintenance Dose of Interferon Alfa After Imatinib Discontinuation in Patients with Chronic Myeloid Leukemia
 1685 Frequency of Molecular Monitoring Correlates with Long Term Outcomes in Chronic Phase Chronic Myelogenous Leukemia Treated with First-Line Imatinib: Results of a Community Survey
 1686 Incidence of Hyperglycemia by 3 Years in Patients (Pts) with Newly Diagnosed Chronic Myeloid Leukemia in Chronic Phase (CML-CP) Treated with Nilotinib (NIL) or Imatinib (IM) in ENESTnd
 1687 Biphenotypic Blast Phase (B-BP) of Chronic Myeloid Leukemia (CML): A Single Institution Experience
 1688 Effects of Tyrosine Kinase Inhibitors On Spermatogenesis and Pituitary Gonadal Axis in Males with Chronic Myeloid Leukemia
 1689 Chronic Myeloid Leukemia Philadelphia Chromosome Positive (CML Ph+) with Imatinib (IM) Intolerance or Resistant Disease. Response to Thalidomide (TALI) Treatment. Preliminary Report of a Serial of Cases with Encouraging Results
 1690 A Sensitive Replicate RQ-PCR of BCR ABL Transcripts Suggests That A Large Portion of Long Term Post Allogeneic SCT CML Patients Are in Deep MR and May Therefore Be Cured From Their Disease
 1691 Restrospective Multicenter Study Evaluating Efficacy and Safety of Dasatinib and Nilotinib in Patients with Imatinib-Resistant or – intolerant Chronic Myeloid Leukemia in Chronic Phase: Korean CML Working Party
 1692 BCR-ABL1 kinase domain mutations may persist sub-clonally for many years, and may be reselected and lead to resistance to subsequent tyrosine kinase inhibitors
 1693 The Outcome of Patients (pts) with Chronic Myeloid Leukemia (CML) Treated with Imatinib Outside of a Clinical Trial or On a Clinical Trial At a Single Institution

13 Therapy: Poster II

- 2781 Understanding United States (US) Treatment Practices for the Management of Chronic Myeloid Leukemia (CML) in Clinical Practice: A US Subgroup Analysis of the WORLD CML Registry
- 2782 Functional Characterization of T- and NK-Cells in Chronic Myeloid Leukemia Patients Treated with Interferon- α Monotherapy
- 2783 Serum Trypsin Is a Strong Predictive Biomarker That Improves Prognostication in Ph+ Chronic Myeloid Leukemia
- 2784 Delayed Cytogenetic Response and Reduced Rate of Major Molecular Response Associated to Increased Body Mass Index At Baseline in Chronic Phase Chronic Myeloid Leukemia Patients Treated with Imatinib
- 2785 RNA Expression and Polymorphisms in Imatinib Influx and Efflux Transporters Influence Molecular Response to Imatinib Therapy in Newly Diagnosed Patients with Chronic Myeloid Leukemia
- 2786 The CML-Microvesicles Are Enriched with Mirnas Regulating MAPK Signaling Pathway
- 2787 Long-Term Follow-up of Ongoing Patients in 2 Studies of Omacetaxine Mepesuccinate for Chronic Myeloid Leukemia
- 2788 Detailed Investigation On Characteristics of Japanese Patients with Chronic Phase CML Who Achieved a Durable CMR After Discontinuation of Imatinib – an Updated Result of the Keio STIM Study
- 2789 Can Targeted Therapy for CML Still Learn From Transplant? Using Post-transplant RQ-PCR monitoring to Clarify the Importance of the Depth of Molecular Remission On the Risk of Subsequent Relapse
- 2790 High BCR-ABL Levels At Diagnosis Are Associated with Unfavorable Responses to Imatinib Mesylate
- 2791 Selective Targeting of CML Progenitor/Stem Cells by the Class 1 Histone Deacetylase (HDAC) Inhibitor MS275 in Combination with Imatinib
- 2792 Benefits of Early Switching From Imatinib to a Second-Generation Tyrosine Kinase Inhibitor Following 12 Month Complete Cytogenetic Response Failure: A Chart Review Analysis
- 2793 Baseline Predictors of Response to Bosutinib in Patients with Chronic Phase Chronic Myeloid Leukemia Following Resistance or Intolerance to Imatinib Plus Dasatinib and/or Nilotinib
- 2794 Comparison of Sokal, Hasford and EUTOS Scores in Terms of Long-Term Treatment Outcome According to the Risks in Each Prognostic Model: A Single Center Data Analyzed in 255 Early Chronic Phase Chronic Myeloid Leukemia Patients Treated with Frontline Imatinib Mesylate
- 2795 Analysis of Bone Marrow Microenvironment Factors As Early Markers of Response in Patients with Newly Diagnosed Bcr-Abl Positive CML in Chronic Phase Treated with Nilotinib
- 2796 Comparison of Disease Outcomes for Patients with Chronic Myelogenous Leukemia in Chronic Phase Switched to Nilotinib or Dasatinib As Second-Line Therapy
- 2797 Cytogenetic and Molecular Responses At 3 Months Are Associated with A Better Outcome in Early Chronic Phase (ECP) Chronic Myeloid Leukemia (CML) Patients Treated with Nilotinib
- 2798 Assessment of Early Cytogenetic Response As a Predictor of Long-Term Clinical Outcomes in a Phase 1/2 Study of Bosutinib in Chronic Phase CML
- 2799 Higher Than Expected Incidence of Chronic Myeloid Leukemia in Solid Organ Transplant Recipients

14 Therapy: Poster III

- 3740 Outcomes in Patients with Chronic Myeloid Leukemia (CML) Who Are Treated with Imatinib As Front Line Therapy
- 3741 The International Registry for Chronic Myeloid Leukemia (CML) in Children and Adolescents (I-CML-Ped-Study): Objectives and Preliminary Results
- 3742 Long-Term Follow-up of Chronic Phase Chronic Myeloid Leukemia Patients Who Failed Interferon Alpha and Switched to Imatinib
- 3743 Long-Term Anti-Leukemic Activity of Ponatinib in Patients with Philadelphia Chromosome-Positive Leukemia: Updated Results from an Ongoing Phase 1 Study
- 3744 Fingolimod (FTY720) Overcomes the Resistance to Tyrosine Kinase Inhibitors Via Dual Activation of BIM and BID in Chronic Myelogenous Leukemia
- 3745 Activation Levels of Natural Killer Cells and CD8⁺ T Cells Correlate Highly with Sustained Complete Molecular Response After Discontinuation of Imatinib in Chronic Myeloid Leukemia Patients
- 3746 Secondary Malignancies in CML Patients – Data From the German CML Study IV
- 3747 Multivariate Analyses of the Clinical and Molecular Parameters Associated with Efficacy and Safety in Patients with Chronic Myeloid Leukemia (CML) and Philadelphia Chromosome-Positive Acute Lymphoblastic Leukemia (Ph+ ALL) Treated with Ponatinib in the PACE Trial
- 3748 Prognostic Factors Identified Via a Cox Proportional Hazard Cure Model Explain Survival Differences After Allogeneic Hematopoietic Stem Cell Transplantation (HSCT) in Chronic Myeloid Leukemia (CML) Between Two Consecutive Patient Cohorts
- 3749 Efficacy and Safety of Ponatinib According to Prior Approved Tyrosine Kinase Inhibitor (TKI) Therapy in Patients with Chronic Myeloid Leukemia in Chronic Phase (CP-CML): Results From the PACE Trial
- 3750 First-Line Treatment and Management of Chronic Myeloid Leukemia (CML) in Clinical Practice: Update of > 1800 Patients (Pts) in the WORLD CML Registry
- 3751 Correlation Between Imatinib Trough Concentration and Long-Term Tolerability in CML Patients
- 3752 Impact of Treatment with Tyrosine Kinase Inhibitors (TKIs) On Blood Levels of Growth Hormone-Related Parameters, Testosterone, and Inhibin B in Juvenile Rats and Pediatric Patients with Chronic Myeloid Leukemia (CML)
- 3753 Blast Phase Chronic Myeloid Leukemia: A Pooled Analysis of Subcutaneous Omacetaxine Mepesuccinate in Treatment-Resistant Patients
- 3754 Achieving a Complete Molecular Remission Under Imatinib Therapy Is Associated with a Better Outcome in Chronic Phase Chronic Myeloid Leukemia Patients On Imatinib Frontline Therapy
- 3755 Chronic Myelogenous Leukemia Patients with Highly Nilotinib-Resistant BCR-ABL Mutations Demonstrate Similar Efficacy to Dasatinib, but Have a Higher Likelihood of Developing New Mutations in the Event of Clinical Resistance
- 3756 Analysis of Clinical Arterial and Metabolic Parameters in Chronic Phase CML Patients On Nilotinib in a Single Center Cohort
- 3757 Disease Patterns for Patients (pts) with Chronic Myeloid Leukemia (CML) That Have BCR-ABL Transcript Levels > 10% At 3 Month of Therapy with Tyrosine Kinase Inhibitors (TKIs)
- 3758 The EUTOS Score Is Highly Predictive for Clinical Outcome and Survival in Asian Patients with Early Chronic Phase Chronic Myeloid Leukemia Treated with Imatinib
- 3759 EUTOS Score Is Also Valid in CML Patients Not Involved in Clinical Studies
- 3760 Is Major Molecular Response a Safe Haven Against Blast Crisis in Cml Patients Treated with Imatinib?
- 3761 Absolute BCR-ABL Transcript Levels At 3 Months but Not At Diagnosis Predict Survival of Chronic Myeloid Leukemia (CML) Patients On Imatinib Therapy
- 3762 A Good Adherence to ELN 09 Recommendations in Chronic Myeloid Leukemia (CML) Treatment with Imatinib, Is Associated with Better Outcomes in Patients Treated Outside Clinical Trials
- 3763 Molecular Responses with Ponatinib in Patients with Philadelphia Chromosome Positive (Ph+) Leukemia: Results From the PACE

Trial

3764 Outcomes of Chronic Myeloid Leukemia (CML) Patients Who Stopped Second Generation Tyrosine Kinase Inhibitors (2GTKIs) As Second Line Treatment. Results of the CML Spanish National Registry (RELMC)
3765 Targeting the Genetic Resistance of JAK2 and BCR/ABL by TG101348
3766 Hypercvad Plus Imatinib or Dasatinib for Patients with Lymphoid Blastic Phase of Chronic Myeloid Leukemia
3767 Treatment Results and Malignant Complications in Patients on Long-Term Treatment with Tyrosine Kinase Inhibitors(TKIs) for Chronic Myeloid Leukemia(CML)
3768 Switching to a Second Generation TKI in Chronic Myeloid Leukemia Patients with Late Suboptimal Response with Imatinib Obtained Better Molecular Responses That the "Watch and Wait" Approach. an Experience of a Multicenter Registry in Patients Outside Clinical Trials
3769 Incidence of Late Chronic Anemia in Newly Diagnosed Patients with Chronic Myelogenous Leukemia Responsive to Imatinib
3770 Pharmacologic Monitoring of Dasatinib As First Line Therapy in Newly Diagnosed Chronic Phase Chronic Myelogenous Leukemia (CP-CML) Identifies Patients At Higher Risk of Pleural Effusion: A Sub-Analysis of the OPTIM-Dasatinib Trial
3771 Early Switch to Nilotinib Does Not Overcome the Adverse Outcome for CML Patients Failing to Achieve Early Molecular Response On Imatinib, Despite Excellent Overall Outcomes in the TIDEL II Trial
3772 Trends in All-Cause Mortality Among Older Patients with CML: A SEER Database Analysis
3773 Higher Incidence of Chronic Myeloid Leukemia (CML) Among Blacks Compared to Whites in the Post-Imatinib Period (2002-2009) Corresponds with Worse Survival Observed within the Surveillance, Epidemiology and End Results 18 Registries
3774 Results of Second Generation Tyrosine Kinase Inhibitor Frontline Therapy in Chronic Myeloid Leukemia with Variant Philadelphia Chromosome
3775 Nilotinib Versus High-Dose Imatinib in Early Chronic Phase CML Patients Who Have Suboptimal Molecular Responses to Standard-Dose Imatinib: Updated Data From RE-Nice Study
3776 High Risk of Error in Categorizing Treatment Response in Individual CML Patients by Standard Cytogenetic Analysis or PCR Assay with High CV: A Bayesian Analysis
3777 Clarithromycin Enhances tyrosine Kinase Inhibitor – Induced Cell Death by Inhibition of Late Stage Autophagy in Patients with Chronic Myeloid Leukemia At Diagnosis or with Resistant Stage of Disease
3778 EUTOS Score Identifies Cases with Poor Outcome in Patients with Early Chronic Phase Chronic Myeloid Leukemia, Though Not Predictive for Optimal Response to Imatinib
3779 Bosutinib As Therapy for Chronic Phase Chronic Myeloid Leukemia Following Resistance or Intolerance to Imatinib: 36-Month Minimum Follow-up Update
3780 Trends in Chronic Myeloid Leukemia Survival in the United States From 1975-2009
3781 Response to Frontline Therapy with Second Generation Tyrosine Kinase Inhibitors in Chronic Myeloid Leukemia: Analysis of Outcome for b3a2 Vs. b2a2 Fusion Transcripts
3782 Change in Chronic Low-Grade Nonhematologic Adverse Events (AEs) and Quality of Life (QoL) in Adult Patients (pts) with Philadelphia Chromosome–Positive (Ph+) Chronic Myeloid Leukemia in Chronic Phase (CML-CP) Switched From Imatinib (IM) to Nilotinib (NIL)
3783 Patient (Pt)-Driven Discontinuation of Tyrosine Kinase Inhibitor Theray in Chronic Phase Chronic Myeloid Leukemia (CML) - Single Institution Experience
3784 Five-Year Results of Nilotinib 400 Mg BID in Early Chronic Phase Chronic Myeloid Leukemia (CML): High Rate of Deep Molecular Response - Update of the Gimema CML WP Trial CML0307
3785 Bosutinib As Therapy for Chronic Phase Chronic Myeloid Leukemia Following Failure with Imatinib Plus Dasatinib and/or Nilotinib: 24-Month Minimum Follow-up Update
3786 Prognostic Factors in the Blastic and Accelerated Phase of Patients with Chronic Myeloid Leukemia (CML) in the Tyrosine Kinase Inhibitor (TKI) Era
3787 Early Assessment of Molecular Response in Chronic Myeloid Leukemia Patients On Dasatinib After Imatinib Failure Identify Patients with Poor Cytogenetic and Molecular Responses
3788 Association Between CYP3A4 Polymorphisms, Trough Imatinib Plasma Concentration and Cytogenetic Response in Chronic Phase Chronic Myeloid Leukemia (CML-CP)

15 All CML abstracts numbers

3, 31, 33, 34, 35, 36, 67, 68, 70, 163, 164, 166, 167, 168, 520, 691, 693, 694, 695, 696, 759, 875, 907, 909, 911, 913, 914, 915, 917, 1215, 1234, 1339, 1360, 1415, 1662, 1663, 1664, 1667, 1668, 1669, 1670, 1672, 1673, 1674, 1675, 1676, 1677, 1678, 1679, 1680, 1681, 1682, 1683, 1685, 1686, 1687, 1688, 1689, 1690, 1691, 1693, 1712, 1903, 1997, 2001, 2066, 2404, 2442, 2445, 2459, 2465, 2495, 2552, 2555, 2769, 2770, 2772, 2774, 2775, 2776, 2777, 2780, 2781, 2782, 2783, 2784, 2787, 2788, 2789, 2790, 2792, 2794, 2795, 2796, 2797, 2799, 2832, 3129, 3166, 3179, 3497, 3726, 3727, 3728, 3729, 3730, 3733, 3735, 3738, 3740, 3741, 3742, 3743, 3744, 3745, 3746, 3747, 3749, 3750, 3751, 3752, 3753, 3754, 3755, 3756, 3757, 3758, 3759, 3760, 3761, 3762, 3763, 3764, 3766, 3767, 3768, 3769, 3770, 3771, 3772, 3773, 3774, 3776, 3777, 3778, 3780, 3781, 3782, 3783, 3784, 3786, 3787, 3788, 4234, 4236, 4237, 4260, 4274, 4279