Abstracts and notes on CML presentations
ASH 2009 New Orleans

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1 Highlights

I have not aimed to review all the abstracts, rather pick up some key themes. I have focused mainly on the oral presentations (33 of them) and clinically relevant studies. Complete abstracts are included for all oral presentations and for some of them I've added notes from the presentations. 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/2009/webprogram/start.html

This was quite an important anniversary for CML treatment. It was in New Orleans in 1999 that Brian Druker first presented data on STI571 (now Imatinib or Glivec). Bud Romine, the first ever CML patient treated with imatinib, took his first capsule in June 1998. The CML educational session presented an overview of what's been achieved over the last 10 years and where we are up to with treatment and monitoring in 2009.

If I had to choose only **three important themes** they would be:

- First release of the nilotinib 'up front' phase 3 study from Novartis [LBA 1].

- The maturing phase 3 studies with higher doses of imatinib and combination with IFN [337, 339, 340].

- Stopping imatinib in PCR-negative patients – what's the latest? [859]

I'll expand on these in the first three headings below.

<u>First release of the Novartis nilotinib 1st line data</u>

The one-year response data from the Novartis ENESTnd Trial were presented. Involving 846 patients, this was a 3 arm study: nilot 300 bid, 400 bid, and imatinb 400 od – 1:1:1 randomisation. At 12 months MMR rates in the three arms (in order indicated above) were 44%, 43% and 22%. CCR rates were 80, 78, 65%.

So it seems that nilotinib is producing better early responses than imatinib – perhaps not a surprise but the first time this has been demonstrated. One slightly odd thing was the 'low' rate of MMR in the imatinib arm at 22%: in the TOPS study [337] at 12 months the MMR rate was 40%. These are not directly comparable of course but it is still a large difference.

This study is quite exciting but very preliminary and in this authors' view does not warrant a change in first line therapy just yet. We have seen a number of studies, the TOPS study ([337], see 4.1) is a good example, where there were significant differences between arms early on but with further follow up these differences disappeared and no significant difference in survival was seen. Robust long term studies are required to see whether these early differences in MMR are maintained and whether survival improvements with the new drug can be achieved without excessive toxicity. Expect similar dasatinib data to be released at ASCO in 2010.

As a side note, the SPIRIT team have lobbied to have nilotinib included in SPIRIT 2, so far without success. It would be very timely now to perform an imatinib vs dasatinib vs nilotinib comparison and perhaps NICE will demand this in due course. Watch this space…

There was an update on the MD Anderson single arm first line use of second generation TKIs: dasatinib [338] and nilotinib [341]. The data for both continue to look promising for both with, I think, little to choose between them at this stage. There wasn't much (anything?) on second line dasatinib and nilotinib, a topic close to the hearts of Brit haematologists in view of recent NICE ACD.

Summary: ENESTnd study

In newly-diagnosed patients responses (molecular and cytogenetic) at one year seem to be better with nilotinib than imatinib. The data are very immature and further follow up is required, particularly evaluating survival, before a change in standard practice could be introduced.

The maturing phase 3 imatinib/IFN dose studies

So now I'm a bit confused. There are three large studies that have been addressing the question of whether higher-dose imatinib or imatinib plus IFN produces a better outcome. The **French SPIRIT study** was the first to release data at ASH last year and the message is the same this year ([340], see 4.4.) Originally a 4 arm study (Imat 400, 600, imat plus Ara-C and imat plus IFN) two of the arms were dropped to leave a study comparing imat 400 with imat 400 plus PEG IFN. 695 patients have been recruited. The French contend that the rate of major molecular response (MMR) is significantly higher at 2 years with imat plus IFN than with imat alone. 71% vs 48%. There was no significant difference between the other arms.

The **German** study group have released their comparative data from the SPIRIT-like **CML IV** study involving 1022 patients ([339] see 4.3). The message is different: they see no significant superiority (MMR) in their imatinib plus IFN arm but do see significant superiority for 800 vs 400 imatinib. Neither the French nor German studies show any differences in OS or PFS in any of their treatment groups.

So, just to confuse things a little more… The **TOPS** study (Novartis) in 476 patients ([337], see 4.1) has shown no significant difference in MMR rate between 400mg (54%) and 800mg (51%) and no difference in CCR either – 76% vs 76% - yes, identical. So for now, the jury's out. Given the additional toxicity of IFN and higher-dose imatinib there is no compelling case for suggesting that initial treatment should be more than imatinib 400. The UK SPIRIT 1 study closed with 285 patients enrolled: we should have sufficiently mature data to submit to ASH in 2010.

Summary: phase 3 studies

- French study shows 24 month MMR benefit in adding IFN, German study does not.
- German study shows 24 month MMR benefit of 800 vs 400 imatinib, TOPS study does not.
- No study has shown a *survival* advantage for any intervention more than imatinib 400.

Imatinib: what's the latest on stopping the drug? Anyone 'cured'??

I think this is a really important theme and congratulations must go to our French colleagues, led by Mahon and Rousselot but taking these studies forward. It's not easy to convince patients or their doctors to stop imatinib if they don't have many side effects, at least in the UK. Abstract [859] provided an update on the French 'STIM' (stop imatinib) study. 69 patients were enrolled who had been PCR negative (not low level in contrast to a German study) for at least 2 years. Approximately 50% of patients remained PCR negative and there are now patients 4 to 5 years off treatment with no recurrent disease in France and also in a similar study in Australia. Should we be looking at this more systematically in the UK? There will be a European 'stop' study coming along

which should hopefully evaluate stopping the newer TKIs as well as imatinib. Until more time passes and more patients studied I'm not sure that we can claim 'cure' as yet but this now seems possible – a concept I don't think we would have considered possible only a few years ago.

Summary: stopping imatinib.

In patients who have been PCR negative for 2 years imatinib can be safely stopped:
- 50% patients will remain PCR negative
- 50% patient will become PCR positive (most within 4-6 months) but they respond again to treatment so it appears nothing is lost.

Where have all the new drugs gone?

One thing that struck me this year was that many of the 'promising' new CML drugs seem to have fallen by the wayside with a couple of exceptions. This shouldn't come as a surprise: CML is a rare disease and most patients do fine on imatinib. T315I-specific inhibitors, for example, are expensive to develop and have a very small market: I still have doubts that they will be viable in the future but here are the contenders for 'next generation' drugs that are in the running at present.

AP24534 seems to be staying the distance and is being used in T315I patients [643]. 20 chronic phase patients who had failed TKIs were enrolled including 7 CP patients with T315I. In CP, there was a 45% MMR rate and 25% CCR. In the 7 patients with T315I, there were 3 MCRs including 2 CCRs. Not bad, but I do wonder whether such a drug will stay the distance, get licensed and make it into clinical practice as 315 is still pretty rare.

PHA739358 is now known as danusertib [864] and the is an ongoing clinical study.

Omacetaxine (not a TKI…) is struggling on but I have to say that it doesn't look too impressive to me [861].

XL228 (Exelixis) and **DCC2036** (Deciphera) seems to have gone quiet. They were both presented at ASH last year but nothing this year.

FTY720 doesn't seem to have come into clinical studies as yet [3259] but perhaps has some promise.

INNO406 (Innovive – bought out by CytRx in 2008) seems to have vanished and MK0457 (Merck) is no longer being clinically developed.

LBH589, a HDAC inhibitor, is being used in patients with minimal disease to 'mop up' the remainder [2194]. Other *in vitro* studies of HDAC inhibitors are ongoing [190].

If you have CML and take **simvastatin**, it may be good for you in more ways than one [2202].

Other interesting snippets…

- Be careful with mutation sensitivity tables [510]. Errors of normalization can mislead and *in vitro* sensitivity may not be reflected in clinical response. More correlation of mutations and therapy with clinical outcome required.

- Paediatric CML is of course rare but now at least there are some data available [342, 863]. Imatinib seems to be appropriate first line therapy, relegating transplant to second line. The drug does seem to slow growth in kids.

- IRIS plods along. 7 year follow up, all is well although 2/3 patients are off drug [1126].

- BCR-ABL and JAK-2, the missing link? JAK-2 seems to phosphorylate tyrosine 177 of BCR-ABL [39] and it's function maybe required to maintain the stability of BCR-ABL [2170]. Anyone for a TKI and JAK-2 inhibitor in combination?!

2 New Molecular Mechanisms in CML [37-42]

2.1. **[37] The Bcr-Abl SH2-Kinase Domain Interface Is Critical for Leukemogenesis and An Additional Therapeutic Target in CML.** *Hantschel*. We previously showed that the Abl SH2 domain is an allosteric activator of c-Abl tyrosine kinase activity and substrate phosphorylation (Filippakopoulos et al. (2008) Cell 134(5), 793-803). This effect is exerted directly by docking of the SH2 domain onto the N-lobe of the kinase domain in the active conformation of c-Abl. We also showed that the same structural mechanism is a critical factor for full activation of the oncogenic fusion kinase Bcr-Abl. Disruption of binding of the SH2 domain to the kinase domain in Bcr-Abl by the Ile164Glu mutation in the SH2 domain, led to a strong reduction in in vitro tyrosine kinase activity and Bcr-Abl autophosphorylation. Unexpectedly, we observed a differential attenuation of downstream signaling pathways upon disruption of the SH2-kinase domain interface, indicating different activation thresholds of Bcr-Abl downstream signaling pathways. Here, we show that disrupting the SH2-kinase domain interface abrogates the transforming capacity of Bcr-Abl. Cells expressing the Bcr-Abl Ile164Glu mutant were unable to generate cytokine-independent colonies in vitro. Furthermore, mice transplanted with Bcr-Abl Ile164Glu expressing bone marrow cells did not develop the characteristic MPD-like disease that is caused by wild-type Bcr-Abl. Mice that received Bcr-Abl Ile164Glu cells showed normal survival, blood counts and histology after more than 100 days post-transplant, despite the presence of Bcr-Abl Ile164Glu-expressing cells in all blood lineages. This shows that the formation of the SH2-kinase domain interface is strictly necessary for Bcr-Abl to cause CML. Together with our data that show sensitization to imatinib inhibition of Bcr-Abl Ile164Glu as compared to Bcr-Abl wild-type, this argues for the SH2-kinase domain interface as an additional drug target on Bcr-Abl that may synergize with tyrosine kinase inhibitors and may be useful to inhibit tyrosine kinase inhibitor resistant Bcr-Abl clones. To address possibilities to interfere with the SH2-kinase domain interface, we are using an engineered binding protein that binds to the Abl SH2 domain with high-affinity and specificity and supposedly disrupts the interface with the kinase domain, resulting in a decrease in Bcr-Abl kinase activity. In conclusion, we provide strong evidence that the structural positioning of the SH2 domain is a crucial factor for constitutive activity, signal transduction and leukemogenicity of Bcr-Abl. Besides oligomerization via the N-terminal coiled-coiled domain and loss of the auto-inhibitory N-terminal myristoyl group, the proper positioning of the SH2 domain appears to be another critical factor that is required for constitutive activation of Bcr-Abl. Inhibitors of the SH2-kinase domain interface of Bcr-Abl may comprise alternative or additional points of pharmacological intervention for the treatment of imatinib-sensitive or -resistant CML or Ph+ acute lymphocytic leukemia.

2.2. **[38] A Novel AHI-1-BCR-ABL-JAK2 Interaction Complex Mediates Cellular Resistance to Tyrosine Kinase Inhibitors in CML.** *DeGeer*. The molecular signature of chronic myeloid leukemia (CML) is the BCR-ABL fusion gene originating in a multipotent hematopoietic stem cell. The BCR-ABL oncoprotein (p210BCR-ABL) has constitutively elevated tyrosine kinase activity that perturbs several signalling cascades, including the PI3K/AKT, JAK2/STAT5, NF-kB, and RAS/MAPK pathways. The current first line treatment for CML is the tyrosine kinase inhibitor imatinib mesylate (IM) that induces clinical remission in most chronic phase CML patients. However, early relapses and IM-resistant disease have emerged and are frequently associated with mutations in the BCR-ABL kinase domain. Our recent studies indicate that CML stem cells are less responsive to IM and other tyrosine kinase inhibitors and are critical target population for IM resistance. It is therefore critical to identify other therapies that target CML stem cells to prevent acquisition of resistance. One candidate target is AHI-1 (Abelson helper integration site 1), a recently discovered oncogene that is deregulated in primary leukemic stem cells from CML patients. AHI-1 contains several domains indicative of signalling functions, including an SH3 and a WD40-repeat domain. We have recently identified a novel AHI-1-BCR-ABL-JAK2 interaction complex that modulates BCR-ABL transforming activity both in vitro and in vivo and play a key role in the IM response/resistance of primary CML stem/progenitor cells. To investigate AHI-1's involvement in mediating this cellular resistance to IM and to test the comparative ability of new ABL and JAK2 inhibitors to inhibit this complex in CML cells, AHI-1 was either stably overexpressed in K562 cells by transduction of EF1a-AHI-1-IRES-YFP lentivirus or suppressed in K562 cells using RNA interference. Interestingly, an increase in cellular proliferation and colony formation and a decrease in apoptosis were observed in the presence of 1, 5 and 10 uM of IM when AHI-1 was overexpressed. Survival of these cells was similar to IM resistant K562 cells, which are highly resistant to IM in vitro and display higher AHI-1 protein expression than parental K562 cells. Suppression of AHI-1 had the opposite effect, with cells displaying heightened sensitivity to IM at concentrations as low as 1 uM. Phosphorylation and total protein expression levels of several proteins known to be involved in BCR-ABL signalling, including JAK2, STAT5, MAPK, SRC, AKT and NF-kB (P105, P50, and P65 subunits), were quantified by Western blot analysis. Elevated phosphorylation and total protein expression levels of several of these proteins were observed when AHI-1 was overexpresessed, in particular in the JAK2/STAT5 pathway and especially in the presence of Interleukin 3. Due to the strong effects AHI-1 had on this signalling cascade, we next inhibited JAK2 activity using a selective JAK2 inhibitor, TG101209, that is highly effective against the V617F mutation and inhibits JAK2 and STAT5 activities in polycythemia vera progenitor cells. AHI-1 overexpressing cells showed reduced proliferation and colony formation when treated with IM and TG101209 in combination compared to either IM or TG101209 alone. Interestingly, treatment with IM (5 uM) or dasatinib (150 nM, DA) in combination with TG101209 (100 nM) resulted in greater inhibition (81% and 85%) of CD34+ CML stem/progenitor cells from IM nonresponders (n=4), compared to the same cells treated with a combination of IM and DA (~60%, $p<0.05$), as measured by colony-forming cell assays. CFSE tracking analysis of cell division in these cells further demonstrated additive antiproliferative activity as a result of combined ABL and JAK2 inhibitors. These results suggest that targeting both BCR-ABL and JAK2 activities may be a potential therapeutic option for IM resistant patients.

2.3. **[39] Jak2 Phosphorylates Tyr 177 of Bcr-Abl Activating the Ras and PI-3 Kinase Pathways and Maintains Functional Levels of Bcr-Abl in Chronic Myelogenous Leukemia.** *Samanta*. We have shown that Jak2 is activated in

Bcr-Abl+ hematopoietic cells. Our findings indicate that Jak2 kinase inhibition overcomes drug-resistance in CML cells (Samanta et al., 2006). Recently, we showed that Jak2 inhibition deactivates the Lyn kinase in Bcr-Abl+ cells by inhibiting SET expression; SET inhibits the PP2A-SHP1 (Samanta et al., 2009). Because of the critical effects of Jak2 in Bcr-Abl+ cells, we explored the mechanism of Jak2 action in CML cells. Bcr-Abl is known to be phosphorylated on a number of tyrosine residues including tyrosine 177. Phosphorylation of Tyr 177 leads to binding to Grb2, activation of the SOS-Ras pathway, and activation of the PI-3 kinase through the Grb2-Gab2 pathway. Here, we show that Jak2 and not Bcr-Abl phosphorylates Tyr 177 of Bcr-Abl. Tyr 177 of Bcr-Abl (YVNV) has the Jak2 consensus target sequence (YxxV/L/I) (Argetsinger et al., 2004) as do a number of other Tyr residues in the Bcr portion of Bcr-Abl (b3a2). Inhibition of Jak2 by a specific Jak2 kinase inhibitor TG101209 (TargeGen Inc., San Diego, CA) and a new Jak2 kinase inhibitor (WP1193, synthesized by Dr. Priebe) in Bcr-Abl+ cells, but not inhibition of Bcr-Abl by imatinib mesylate (IM), rapidly reduced phosphorylation of Tyr 177 of Bcr-Abl in IM-sensitive and –resistant cell lines including K562, K562-R, Bcr-Abl+ 32D, Bcr-Abl+ BaF3, Bcr-Abl+T315I BaF3 cells and cells from blast crisis CML patients. Jak2 inhibitors but not IM also strongly inhibited the phosphorylation of a synthetic Bcr peptide containing Tyr 177 (and the surrounding Bcr sequences) catalyzed by either recombinant purified Jak2 or Jak2 immune complexes. Jak2 inhibition of Bcr-Abl+ 32D cells rapidly reduced Grb2 binding to Bcr-Abl, and diminished activation of Ras and PI-3 kinase pathways within two h. Importantly, knockdown of Jak2 with Jak2-specific siRNA also reduced levels of pTyr 177 Bcr-Abl and total Bcr-Abl protein. Rescue experiments reversed Jak2 knockdown and stimulated pTyr 177 levels. In whole cells the total levels of the Bcr-Abl protein also decreased upon Jak2 inhibition with the above chemical inhibitors but at a rate slower than the rate observed in the inhibition of the Tyr 177 phosphorylation of Bcr-Abl. In kinase assays with Bcr-Abl network complexes from Bcr-Abl+ cells, phosphorylation of Tyr 177 of Bcr-Abl was drastically reduced by Jak2 inhibition but Bcr-Abl protein levels remained stable. Reduction of Bcr-Abl protein levels in Bcr-Abl+ cells by Jak2 inhibition also caused a severe reduction of tyrosine phosphorylation of STAT5 and STAT3. We have shown by gel filtration column chromatography of lysates isolated from Bcr-Abl+ cells that Bcr-Abl is physically associated with the IL-3 receptor beta chain, Jak2, Akt and HSP90 in high molecular weight complexes. GSK3 , STAT5 and STAT3 also emerge from the column in the high molecular weight fractions and suggested that several high molecular weight complexes may exist. Nevertheless, incubation of Bcr-Abl+ cells with WP1193 caused disruption of these large molecular network complexes within three h. Nude mice, injected subcutaneously with IM-resistant K562-R cells developed solid tumors, which were reduced in weight by i.p. injection of WP1193 every 48 h at 30 mg/Kg over 10 days. WP1193 at a similar dosage strongly reduced leukemic and tumor effects of Bcr-Abl+ 32D cells expressing T315I Bcr-Abl injected i.v. into nude mice. WP1193 injections into nude mice did not cause significant toxicity over a 10 day period. In summary, Jak2 inhibitors TG101209/WP1193 rapidly reduced levels of pTyr 177 Bcr-Abl, reduced levels of Bcr-Abl protein and induced high levels of apoptosis in IM-sensitive and –resistant Bcr-Abl+ cell lines including T315I cells, CML cell lines, cells from CML patients in blast crisis. In addition Jak2 inhibition reduced tumor formation in mice injected with IM-resistant K562-R and T315I Bcr-Abl+ mouse cells. These findings establish: 1) Jak2 as a major driver of Bcr-Abl signaling pathways in Bcr-Abl+ CML cells, which were previously thought to be activated directly by Bcr-Abl. 2) Although Jak2 is activated by Bcr-Abl, Jak2 is the dominant target molecule in CML and Jak2 becomes even more dominant in CML cells that become resistant to IM and other tyrosine kinase inhibitors. These findings indicate that CML cells, because of the dominant role of Jak2, resemble leukemia cells from Bcr-Abl negative myeloproliferative diseases.

2.4. **[40] GNF-2, An Allosteric BCR-ABL Inhibitor, Identifies a Novel Myristoylation-Mediated Mechanism Regulating the Ability of BCR-ABL to Activate HCK and IGF-1 Signaling.** *Geyer*. Pharmacologic inhibition of BCR-ABL, using clinically active ATP-competitive inhibitors imatinib, nilotinib, and dasatinib, has been used to investigate BCR-ABL kinase activated signaling pathways. However, these agents show cross reactivity with other kinases (e.g. Kit, PDGFR, SRC family members), and their multi-targeted nature complicates assigning biological effects to the inhibition of a specific kinase target. Allosteric kinase inhibitors modulate the catalytic activity of protein kinases by binding to a site distant from the active site and inducing a protein conformation that inhibits kinase activity. These agents show promise as clinical agents and may offer advantages over ATP-competitive inhibitors in studying the function of specific kinases because they exploit binding sites and regulatory mechanisms that are unique to a particular kinase. GNF-2, a mono-selective BCR-ABL inhibitor that targets wild-type BCR-ABL and many clinically relevant imatinib resistant mutants, was recently discovered and provided the first demonstration that c-ABL kinase activity could be modulated by an inhibitor that binds outside the ATP or substrate binding sites. GNF-2 binds to a myristoyl-binding pocket in the C-lobe of the c-ABL kinase domain but its mechanism of inhibiting specific BCR-ABL kinase targets remains unclear. We previously reported that BCR-ABL activates an autocrine IGF-1 pro-survival signaling pathway in CML blast crisis cells through HCK-mediated activation of STAT5b. As GNF-2 is known to inhibit STAT5b phosphorylation, and HCK myristolyation is known to regulate its cellular localization, we hypothesized that GNF-2 inhibits BCR-ABL activation of HCK by binding to the ABL myristoyl-binding pocket and blocking access to the HCK myristoyl moiety. In support of this hypothesis, we now show that GNF-2 inhibits HCK phosphorylation and IGF-1 activation, but not HCK binding to BCR-ABL. To confirm the importance of the HCK myristoyl moiety in HCK activation, we mutated the myristoyl attachment site at position 2 in HCK from glycine to alanine. The mutant G2A HCK still interacted with BCR-ABL in co-immunoprecipitation assays but showed significantly lower levels of phosphorylation compared to wild-type HCK. To confirm that the decrease in phosphorylation was not due to mislocalization of G2A HCK, we mutated the myristoylation binding pocket of BCR-ABL by changing glutamic acid at position 505 to lysine. Similar to G2A HCK, E505K BCR-ABL still interacted with HCK, but the phosphorylation levels of HCK were dramatically reduced. To confirm that the HCK myristoyl moiety directly interacted with the ABL myristoyl-binding pocket, we used fluorescent spectroscopy to measure the ability of a myristoylated peptide corresponding to the six N-terminal amino acids of HCK to displace GNF-2. The fluorescence of GNF-2 is enhanced when it associates with the myristoyl-binding pocket of ABL. Using this assay, we calculated the Kd of GNF-2 to be 180 nM. We then assayed the ability of myristolyated HCK peptide to displace GNF-2 from ABL. We calculated the IC50 of the myristolyated HCK peptide to be 25 µM when ABL was saturated with 300 nM GNF-2. Myristate showed an IC50 of 213 µM, which is ~ 10-fold higher than the myristoylated peptide. No binding was detected between the non-myristoylated peptide and ABL. Together, our study highlights a novel acquired

function resulting from the fusion of BCR to the N-terminus of ABL, which converts the myristoyl-binding pocket in ABL from a negative regulator of kinase activity to an HCK activation motif that activates downstream IGF-1 signaling. These results also reveal the mechanism of action of the mono-selective BCR-ABL inhibitor GNF-2 and highlight the ABL myristoyl-binding pocket as a therapeutic target for inhibiting BCR-ABL activity.

2.5. **[41] Overexpression and Phosphorylation of eIF4E Is Required for Beta-Catenin Activation in Blast Crisis Chronic Myelogenous Leukemia.** *Lim*. Despite the success of tyrosine kinase inhibitors (TKI) in chronic phase (CP) CML, blast crisis (BC) CML remains difficult to control, with most patients eventually developing either primary or secondary TKI-resistance (TKI-R). In addition to TKI-R, myeloid BC GMPs acquire the ability to self-renew, a feature that is mediated by b-catenin activation, and which may contribute to disease persistence. The mechanisms underlying b-catenin activation remain obscure, including its dependence on Bcr-Abl activity. Long-term control of BC CML will require overcoming the mechanisms mediating TKI-R, as well as reversing b-catenin-mediated self-renewal. In this study, we investigated the role of the mRNA translation machinery in mediating b-catenin-mediated self-renewal, since our prior work had implicated aberrant mRNA translation in TKI-R and BC pathophysiology (Ly et al. Cancer Research 2003; Prabhu et al. Oncogene, 2007; Zhang et al. MCB, 2008). First, we showed that primary CD34+ BC cells overexpressed eIF4E, a rate-limiting translation initiation factor, compared to CP cells. We also found that both eIF4E overexpression and phosphorylation at serine 209 (S209) occurred in a Bcr-Abl-independent manner. Next, by using a combination of genetic and biochemical approaches, we showed that forced overexpression of eIF4E was sufficient to activate b-catenin in the K562 BC cell line, as measured by a b-catenin reporter (a 2-fold increase in TOP/FOP ratio) and by an increase in nuclear b-catenin (a 2.5-fold increase in mean fluorescence intensity using immunofluorescence). Importantly, overexpression of an eIF4E S209A mutant did not show increased b-catenin activity, whereas overexpression of a phospho-mimetic S209D mutant did, indicating b-catenin activation was dependent on S209 phosphorylation. Next, we found that siRNA knock-down or small molecule inhibition (CGP57380) of the Mnk 1/2 kinases (which are responsible for in vivo phosphorylation of eIF4E) prevented the increased b-catenin activity induced by eIF4E overexpression. Also, Mnk1/2 inactivation had no effect on b-catenin activity in the S209D mutants. Because recent work has implicated GSK3b inactivation as a contributor to b-catenin activity in BC GMPs (Abrahamsson et al. PNAS, 2009), we repeated these experiments in the presence of the GSK3b inhibitor, BIO. Here, we found that both eIF4E overexpression and BIO treatment cooperated to further augment b-catenin activity (a 3 to 4-fold increase in the TOP/FOP ratio, and 3-fold increase in nuclear b-catenin), and that this was an effect that required eIF4E phosphorylation. Based on these data, we performed biochemical and functional analyses of primary CP and BC CML cells, as well as normal cord blood (CB) progenitors. As reported by others, we found increased nuclear b-catenin in CD34+CD38+ BC cells compared CP or CB cells. Inhibition of Mnk 1/2 was also able to prevent accumulation of nuclear b-catenin in CD34+CD38+ BC cells, as was a known b-catenin inhibitor (CGP049090), but not imatinib. Functionally, Mnk kinase inhibition by CGP57380 (2.5-10 microM) also significantly decreased the ability of BC CFCs to serially replate (>8 weeks) in methylcellulose (by >90%) compared to CB CFCs (25-50%). Finally, since recent work has demonstrated that activation of the serine/threonine phosphatase, PP2A, can impair b-catenin activity and self-renewal in CML stem cells (Neviani et al, ASH Meeting Abstracts, 2008), we tested the ability of the PP2A activator, FTY720, to inhibit b-catenin activity in the context of eIF4E overexpression. We found that while FTY720 was able to inhibit b-catenin activity in cells overexpressing wild-type eIF4E, it could not do so in the presence of the S209D mutants. In summary, our results indicate that: (i) increased expression and phosphorylation of eIF4E at S209 is sufficient to activate b-catenin in CML cells; (ii) GSK3b inactivation and eIF4E phosphorylation cooperate to augment b-catenin activity; (iii) the serial-replating ability of BC CML progenitors is dependent on eIF4E phosphorylation; (iv) dephosphorylation of eIF4E at S209 is important in enabling the PP2A activator, FTY720, to inhibit b-catenin activity. Taken together, our data show that targeting eIF4E function, by modulating the activity of the kinases or phosphatases which regulate S209 phosphorylation, is a novel approach to overcome b-catenin-mediated self-renewal in BC CML cells, but not normal progenitors.

2.6. **[42] Placental Growth Factor: a Novel, Stromal-Derived Target in Human CML.** *Loges*. Although BCR-ABL kinase inhibitors (TKIs) have revolutionized treatment of CML, they do not eradicate disease and treatment resistance can emerge. We recently identified Placental Growth Factor (PlGF) as an additional pathogenic factor in murine BCR-ABL1+ leukemia. PlGF induces proliferation of bone marrow stromal cells and of BCR-ABL1+ cell lines. PlGF is upregulated in blood (PB) and bone marrow (BM) of mice with BCR-ABL1+ leukemia and it's inhibition by a monoclonal antibody (PlGF) significantly prolongs survival of mice bearing BCR-ABL1-unmutated and -T315I mutant blast crisis (BC) CML (Loges et al., Blood 2008; 112: abstract 1094). Now, we have further characterized the role of PlGF in disease progression, the mechanism of action of PlGF and validated PlGF as novel target in human CML. We first quantified PB and BM PlGF protein levels at different time points after inoculation of BCR-ABL1 transduced BM cells and found significantly increasing PlGF levels upon disease progression. In line with those data, PB and BM PlGF protein levels correlated with numbers of GFP-marked leukemia cells (r=0.81; p=<0.001 and r=0.85; p=0.001, respectively). We unraveled that PlGF is almost exclusively expressed by the non-hematopoietic (CD45-), non-leukemic (GFP-) stromal cell compartment. In vitro data indicate specific PlGF upregulation by CD45- BM stromal cells upon co-culture with BCR-ABL1+ leukemia cells, but not in hematopoietic cells or endothelial cells. Hence, PlGF is a stromal target induced by interaction with BCR-ABL+ leukemia cells and significantly correlates with disease burden. Neutralization of PlGF by PlGF and/or its genetic deletion prolong survival of mice bearing BCR-ABL1+ leukemia. Therefore we analyzed the anti-leukemic efficacy of PlGF treatment by measuring GFP+ leukemia cells in the PB of mice at an early time-point (d14) and at end-stage disease (d28). We found a reduction of leukemia cells by 42 and 51% in mice treated with PlGF when compared control mice (p<0.05). Furthermore, we analyzed the effect of PlGF inhibition on the leukemic BM microenvironment. Morphometric analysis of CD31 immunostaining revealed reduced microvessel density (MVD) in PlGF treated mice compared to controls (236+15 vs. 307+17 vessels/mm2; p=0.006). Investigation of reticulin fibers indicated that PlGF reduces the prominent fibrosis of the BM occurring upon disease progression by 30% (p=0.001), which complements the finding that PlGF expands BM derived stromal cells in vitro. Hence, PlGF reduces the leukemic burden and normalizes the abnormal leukemic BM by reducing

hypervascularization and BM fibrosis. To investigate the relevance of PlGF as novel target molecule in human CML, we determined PlGF plasma levels in healthy controls (n=10), untreated patients in Chronic Phase (CP) upon primary diagnosis (n=32) and patients with BC under treatment with different TKIs (n=9). These analyses revealed 2.1-fold upregulation of PlGF in newly diagnosed patients in CP (p<0.0001) and 3.7-fold increase of PlGF levels in patients with BC (p<0.0001) compared to healthy controls. We then investigated a potential relation between PlGF protein and BCR-ABL1 transcript numbers as determined by QRT-PCR in a single centre (n=43 CP, n=2 Accelerated Phase; treatment with different TKIs, imatinib+interferon, or homoharringtonine). We found a significant correlation between PlGF levels and BCR-ABL1 transcript numbers (r=0.45; p=0.0016), indicating that PlGF represents a disease specific target in human CML. Subsequently, we isolated CD34+ cells from healthy donors and from CML patients in CP and BC and determined PlGF expression by QRT-PCR. These analyses revealed that PlGF expression is equally low in leukemia cells as in healthy CD34+ cells. Thus, elevated circulating PlGF is most likely not secreted by leukemia cells, but by stromal cells. To investigate this hypothesis, we isolated adherent BM stromal cells from patients with newly diagnosed CML and compared PlGF expression levels to those in CD34+ leukemia cells. We found that stromal cells express >7-fold more PlGF than leukemia cells (p=0.003), which corroborates our preclinical data and extends the concept that PlGF is primarily produced by stromal cells in CML patients. In summary inhibition of PlGF may serve as a new candidate to be targeted in combination therapies or in TKI refractory CML.

3 Progenitor and Stem Cells in CML [187-192]

3.1. **[187] Foxo Transcription Factor Activity Is Retained in Quiescent Chronic Myeloid Leukaemia Stem Cells and Activated by Tyrosine Kinase Inhibitors to Mediate “induced-quiescence” in More Mature progenitors.** *Cilloni*. First line therapy for CML involves tyrosine kinase inhibitors (TKIs) which can induce rapid cytogenetic responses in the majority of patients in chronic phase (CP), but do not eliminate BCR-ABL transcripts in the majority, suggesting persistence of residual disease. These findings, together with the rapid kinetics of relapse in patients who discontinue TKIs, suggest the presence of a reservoir of TKI-resistant leukaemic stem cells, although the mechanism for TKI-insensitivity of CML stem cells remains unclear. The FoxO family of transcription factors is mainly regulated by PI3K/Akt induced phosphorylation, resulting in nuclear exclusion and degradation. FOXO activity is implicated in maintaining haemopoietic stem cell (HSC) quiescence. Its transcriptional activity in normal HSC results in cell cycle arrest by expression of p27, p130, p21, down-regulation of Cyclin D and protection from oxidative stress. Cell line studies suggest that FOXOs may play a central role in the anti-proliferative effects of TKIs, but their role in primary CML stem cells has not been previously investigated. Methods: Quiescent CML stem cells were isolated phenotypically (Lin-CD34+38-) by FACS from the total CD34+ stem/progenitor cell population and D-FISH analysis performed to determine the percentage of Ph+ cells. The expression levels of Spred1, FOXO1, FOXO3a, FOXO4 and Cyclin D1 were evaluated by Real-Time-PCR. Protein levels and localization were studied by Western blot, immunofluorescence and FACS. EMSA assay was used to evaluate FOXO3a DNA binding activity. K562 cells were transfected with wt FOXO3a and the constitutively active triple mutant (TM) form of FOXO. After transfection, proliferation and apoptosis were tested using incorporation of H3 thymidine and annexin V detection by FACS, respectively. Results: We found that BCR-ABL, through activation of the PI3K/Akt pathway, induces phosphorylation and cytoplasmic localization of FOXO in CD34+ CML cells, thereby blocking its transcriptional activity as demonstrated by EMSA and by the inhibition of FOXO target genes, including Spred1. Incubation with TKIs decreased phosphorylation and induced re-localization of FOXO to the nucleus in CD34+ CML cells, thus restoring FOXO transcriptional activity. This resulted in decreased levels of Cyclin D1 and reduced ROS. Similar effects and a dramatic reduction of cell proliferation, accompanied by significant apoptosis, were observed by forcing the expression of FOXO3a in K562 through transfection of the TM form, confirming the role of FOXO in inducing cell cycle arrest. Incubation experiments using LY294002, rapamycin and TKIs established that the reactivation of FOXO by TKI is mediated by the PI3K/Akt pathway. Interestingly, we found that phosphorylation of FOXO1, 3a and 4 was higher (i.e. cytoplasmic and inactive) in proliferating CD34+38+ CML cells, as compared to more primitive and quiescent CD34+38-90+ CML cells, although the activity of BCR-ABL, measured by analysis of p-CrKL was found to be similar in both populations, indicating that although present, BCR-ABL was not inducing phosphorylation of FOXO in the quiescent population. In this sense the quiescent CML stem cells resembled normal HSC in terms of FOXO regulation. In conclusion our data indicate that TKIs initiate a process in CML stem and progenitor cells that maintains their quiescence and therefore potential resistance to TKIs themselves. The anti-proliferative activity of TKIs against primary CML CD34+ cells is mediated, at least in part, by the re-activation of FOXO1, 3a and 4. BCR-ABL appears to play a different role in more mature progenitor cells compared to primitive quiescent stem cells, suggesting the possibility of an incomplete activity of BCR-ABL at the stem cell level or alternatively, the possibility that FOXO activity at this level provides the dominant signal responsible for intrinsic quiescence.

3.2. **[188] The Tumor Suppressor Role of the Msr1 Gene in Cancer Stem Cells of Chronic Myeloid Leukemia.** *Chen*. We have previously shown that the arachidonate 5-lipoxygenase gene (Alox5) functions as a critical regulator of leukemia stem cells (LSCs) in BCR-ABL-induced chronic myeloid leukemia (CML) in mice (Chen Y, Hu Y, Zhang H, Peng C, Li S. Loss of the Alox5 gene impairs leukemia stem cells and prevents chronic myeloid leukemia. Nature Genetics 41:783-792, 2009). We believe that the Alox5 pathway represents a major molecular network in LSCs. Therefore, we decided to further dissect this pathway by comparing gene expression profiles between wild type and Alox5-/- LSCs from CML mice using the DNA microarray analysis. We identified a small group of candidate genes that were changed in expression in the absence of Alox5. Among these genes, we have identified the Msr1 gene and chosen to test the function of this gene in regulating LSC function, because this gene was up-regulated, indicating that it might play a tumor suppressor role in LSCs. In our CML mouse model, we observed that recipients of BCR-ABL transduced Msr1-/- bone marrow cells developed CML much rapidly than recipients of BCR-ABL transduced wide type bone marrow cells. To test whether this accelerated CML is related to abnormal function of LSCs, we carried out a serial transplantation assay by transferring bone marrow cells from primary recipients of BCR-ABL-transduced wild type or Msr1-/- donor bone marrow cells into secondary and next-generation

of recipient mice to biologically assess the effect of Msr1 on LSCs. BCR-ABL-expressing wild type leukemia cells from bone marrow of CML mice were only able to transfer CML once, whereas BCR-ABL-expressing Msr1-/- leukemia cells were able to transfer lethal CML for five genrations. This observation indicates that BCR-ABL-expressing Msr1-/- LSCs have markedly increased stem cell function. To further compare the stem cell function, we performed the leukemia stem cell competition assay by 1:1 mixing wild type (CD45.1) and Msr1-/- (CD45.2) bone marrow cells from CML mice. At day 25 or 30 after transplantation, more than 60% and 95% of GFP+Gr-1+ cells in peripheral blood of the mice were CD45.2+Msr1-/- myeloid leukemia cells, and all these mice developed CML and died of CML derived from Msr1-/- LSCs. To confirm the tumor suppressor role of Msr1 in CML development, we co-expressed BCR-ABL and Msr1 in MSR1-/- bone marrow cells by retroviral transduction, followed by transplantation of these cells into recipient mice. The ectopically-expressed Msr1 in MSR1-/- bone marrow cells rescued the accelerated CML phenotype, and some recipient mice did not even develop the CML. Together, these results demonstrate that Msr1 plays a tumor suppressor role in LSCs. The Msr1 pathway is a novel molecular network in LSCs, and it will be important to fully study this pathway for developing curative therapeutic strategies for CML.

3.3. **[189] Role of the SIRT1 Deacetylase in Survival and Imatinib Resistance of CML CD34+ Progenitors.** *Li*. Imatinib mesylate (IM) treatment has primarily anti-proliferative effects on CML progenitors, and only modest induction of apoptosis is observed. Quiescent, primitive progenitors are especially insensitive to IM induced apoptosis. Identification and targeting of mechanisms of resistance to IM is required to allow enhanced elimination of residual CML progenitors in IM treated patients. SIRT1 is a NAD+ dependent deacetylase that regulates activity of several proteins involved in stress responses. We have observed significantly increased expression of SIRT1 mRNA and protein in CML compared to normal CD34+ progenitors. Here we investigated the effect of inhibition of SIRT1 expression on growth, survival and IM sensitivity of CML progenitors. CML and normal CD34+ cells were transduced with lentivirus vectors expressing SIRT1 shRNAs (Si-1 or Si-2) or control shRNA (Ctrl). Inhibition of SIRT1 expression in Si-1 (95% inhibition) and Si-2 (80% inhibition) transduced CD34+ cells was confirmed on Western blotting. SIRT1 inhibition resulted in modest increase in apoptosis of CML progenitors (Si-1 16±9%, Si-2 10±5% and Ctrl 8±4% apoptosis), and significantly enhanced sensitivity of CML progenitors to IM (2.5 M) induced apoptosis (Si-1, 27±12%, Si-2, 15±4% and Ctrl, 14±5%, Si-1 versus Ctrl, p=0.04, n=4). SIRT1 inhibition did not induce apoptosis in normal progenitors or increase their sensitivity to IM (Si-1 14±3%, Si-2, 13±4% and Ctrl, 11±2% without IM; and Si-1, 14±2%, Si-2, 12±3% and Ctrl, 12±2% with IM). Importantly SIRT1 inhibition significantly enhanced apoptosis of non-dividing (CFSE bright) CML progenitors treated with IM (Si-1, 49±16% and Ctrl, 33±4%, p=0.02, n=4). SIRT1 knock down inhibited proliferation of CML progenitors measured by CFSE labeling (Si-1, 46±5% and Si-2, 14±3% inhibition versus Ctrl, p<0.01, n=4) and enhanced inhibition of CML progenitors in combination with IM (Si-1, 69±11% and Si-2, 53±12% inhibition versus Ctrl, 44±9%, p<0.05, n=4). Inhibition of normal progenitor proliferation was also seen, but was significantly less than that of CML progenitors (Si-1, 30±6% and Si-2, 8±3% inhibition compared with Ctrl, p<0.05, n=4; in combination with IM, Si-1, 33±5% and Si-2, 9±4% inhibition compared with Ctrl, 8±2% inhibition). SIRT1 inhibition also reduced the number of cells generated in culture from CML progenitors (20-fold reduction in cells generated at day 14 with Si-1 compared to controls). Relatively greater inhibition of myeloid compared with erythroid cell growth was seen (at day 14, CD33+ cells with Si-1 0.42±0.21x106 and Ctrl 1.72 ± 0.61 x106; CD11b+ cells with Si-1 0.32±0.19 x106 and Ctrl 9.46 ±1.33 x106). SIRT-1 inhibition also reduced the number of colonies generated from CML CD34+ cells in methylcellulose progenitor culture by itself (17±6% CFC with Si-1 compared to controls, p=0.005, n=3), and in combination with IM (3±1% CFC with Si-1 compared to 23±8% with Ctrl, p=0.03, n=4). The greater effects of Si-1 compared to Sir-2 shRNA in the above studies suggest that near complete knock-down of SIRT-1 is required to alter CML progenitor proliferation and survival. The above results indicate that the stress response gene SIRT1 contributes to enhanced survival and proliferation of CML progenitors and protects CML progenitors from IM-induced apoptosis. SIRT1 inhibition may provide a novel and effective strategy to eradicate residual CML progenitors in combination with IM.

3.4. **[190] Effective Targeting of Quiescent CML Stem Cells by Histone Deacetylase Inhibitors in Combination with Imatinib Mesylate.** *Zhang*. The BCR-ABL tyrosine kinase inhibitor imatinib mesylate (IM) is highly effective in inducing remissions and improving survival in CML patients but fails to eliminate leukemia stem cells, which remain a potential source of relapse. Quiescent leukemia stem cells resist apoptosis following BCR-ABL kinase inhibition, and other strategies are required for their elimination. Histone deacetylase inhibitors (HDACi) have shown promise in the treatment of several cancers, and it is of particular interest that reports suggest that they are also capable of inducing apoptosis in non-proliferating cells. It is known that the potent pan-HDACi LAQ824 (LAQ) and LBH589 (LBH) can induce apoptosis in CML cell lines and blast crisis CML cells. However, the effect of HDACi on quiescent leukemia stem cells from chronic phase CML patients is not known. Here we investigated the effects of LAQ and LBH, alone and in combination with IM, on CML stem and progenitor cells. CML and normal CD34+ cells were cultured with LAQ (10-100nM) or LBH (25-50nM) alone, IM (1mM) alone, and LAQ or LBH with IM for 96 hours. HDACi treatment effectively enhanced Histone H3 and H4 acetylation in CML CD34+ cells. HDACi treatment by itself induced less apoptosis in CML compared to normal CD34+CD38- primitive and CD34+CD38+ committed progenitors, but was highly effective in inducing apoptosis in CML progenitors when combined with IM. In addition, the combination induced significantly higher apoptosis in CML compared with normal CD34+ cells, and unlike IM, also induced apoptosis in non-dividing cells. Combination treatment also inhibited the proliferation of CML progenitor cells as measured by CFSE and growth of CML CFC in methylcellulose progenitor assays. Treatment of CML CD34+ cells with IM resulted in modest reduction in levels of engraftment in NSG mice. In contrast treatment with LAQ824 plus IM resulted in abrogation of engraftment of BCR-ABL+ CML cells (p<0.001). Significantly less inhibition of normal compared to CML cell engraftment was seen following LAQ824 and IM treatment (p=0.006). We used a transgenic Scl-tTa-BCR-ABL mouse model to investigate the effect of HDACi treatment on CML stem cells in vivo. SCLtTA/BCR-ABL transgenic mice were crossed with GFP transgenic mice to allow tracking of transplanted cells. Recipient mice developed CML-like disease 3-4 weeks after transplantation. Mice were treated with IM (200mg/kg daily by gavage), LBH (30 mg/kg IP 3 times a week), LBH with IM, or vehicle alone (control) for 4 weeks. LBH combined with IM resulted in greater reduction in WBC, neutrophils

and GFP+ cells than LBH or IM alone. Significantly increased apoptosis and a profound reduction of Lin- Sca-1+ Kit+ (LSK) stem cells were seen in mice treated with IM plus LBH (p<0.001) but not IM or LBH alone. LBH plus IM treatment did not significantly inhibit LSK cells in normal mice. BCR-ABL-transgenic mice treated with IM plus LBH demonstrated prolonged leukemia-free survival after discontinuing treatment compared with mice treated with IM or LBH alone (p<0.05). Combined IM and LAQ824 treatment resulted in marked inhibition of the anti-apoptotic protein MCL-1 in CML CD34+ cells (p<0.001). RNAi mediated inhibition of MCL-1 expression resulted in increased apoptosis in CML compared with normal CD34+ cells that was further enhanced by IM treatment. Our results indicate that treatment with HDACi plus IM effectively and selectively targets leukemia stem cells in CML, suggest a potential role for MCL-1 inhibition in HDACi induced apoptosis, and support ongoing clinical trials of LBH combined with IM to eliminate residual leukemia stem cells in IM-treated CML patients.

3.5. **[191] BCR/ABL Dosage Hierarchically and Temporally Influences hnRNP A1, hnRNP K and hnRNP E2 Expression in Hematopoietic Stem and Progenitor Cells.** *Harb.* The molecular mechanism leading to the progression of chronic myelogenous leukemia (CML) from the indolent chronic phase (CML-CP) to the rapidly fatal blast crisis (CML-BC) are still unclear although a plausible assumption is that enhanced expression of BCR/ABL, as that observed in most of patients undergoing progression, represents the factor promoting clonal evolution of CML. Given that a) BCR/ABL levels are increased in the CML-BC stem/leukemia-initiating cell population; b) a causal relationship exists between levels and activity of the BCR/ABL oncoprotein and aberrant mRNA processing, nuclear export and/or translation; and c) molecular and/or pharmacologic interference with the expression and/or activity of the BCR/ABL-regulated RNA binding proteins (hnRNP A1, hnRNP K and hnRNP E2) antagonizes both in vitro and in vivo BCR/ABL leukemogenesis by impairing proliferation, inhibiting survival and/or restoring differentiation of BCR/ABL+ hematopoietic progenitors; we hypothesized that BCR/ABL initiates a hierarchical activation of signals leading to a temporally-organized increase in the expression/function of specific RNA binding proteins (RBPs), and that this represents an essential step for disease progression. To determine whether expression of hnRNP A1, K and E2 is hierarchically regulated by BCR/ABL and at which stage of the CML stem/progenitor cell development it occurs, we first transduced 32Dcl3 cells with the MigR1-BCR/ABL construct and allowed the clones from a 32D-BCR/ABL^{low} cell population to become BCR/ABL^{high}/hnRNP E2^{high} /C/EBPa^{-} within 21 days of culture in the presence of IL-3. Western blots indicate that BCR/ABL-dependent full induction of hnRNP A1 expression precedes that of hnRNP K and E2 which occurs only after BCR/ABL levels and activity increase to levels capable of conferring cytokine-independent growth and differentiation arrest, suggesting that hnRNP A1 may have the role of a "gatekeeper", as it allows the increase of BCR/ABL expression through inhibition of PP2A. This, in turn, will promote disease progression in part by inducing expression and activity of hnRNP K and E2 that, as we previously reported, regulate survival, proliferation and differentiation of CD34^{+} CML-BC progenitors. Notably, we observed a similar pattern of hnRNP induction in the lymphoid BCR/ABL-inducible TonB210157 cells. We also have evidence that differences in hnRNP A1, hnRNP K and hnRNP E2 expression exist between the stem and progenitor cell fractions of BCR/ABL+ primary cells and that they are differentially regulated in leukemic and normal cells. In fact, FACS analysis followed by intracellular protein staining performed on bone marrow- and spleen-derived LSK (Lin^{-}/Sca-1+/c-kit^{+}), common myeloid progenitors (CMPs) (Lin^{-}/Sca-1^{-}/c-kit^{+}/CD34^{+}, FCgRII/III^{dim}) and granulocyte monocyte progenitors (GMPs) (Lin^{-}/Sca-1^{-}/c-kit^{+}/CD34^{+}/FCgRII/III^{bright}) from leukemic SCLtTA-BCR/ABL double-transgenic mice showed that levels of hnRNP A1 were 3 to 5-fold higher in CMP/GMP than LSK cell fractions (LSK<CMP<GMP). By contrast, in non-induced animals, hnRNP A1 expression was overall markedly inhibited (10-fold lower) with respect to leukemic mice and progressively decreased during LSK maturation into GMPs (LSK>CMP>GMP). Likewise, hnRNP K and E2 levels were 2-fold increased in the progenitors compared to the LSK of leukemic animals, whereas an opposite trend in the expression of hnRNP K was observed in the LSK and CMPs/GMPs of non-induced animals. Moreover, hnRNP K levels in the leukemic CMPs/GMPs were 30 to 40-fold higher than those detected in the same cell fractions from non-induced animals. Interestingly, the highest levels of hnRNP A1 and K in the CMP/GMP fractions correlated with the development of a lymphoid blast crisis-like phenotype as determined by the 30% increase in splenic B220^{+}/Mac-1^{+} cells. Altogether these data suggest that hierarchical and temporal changes in the expression of hnRNPs occur upon BCR/ABL transformation in stem and progenitor cells and during disease progression. Furthermore, these results are consistent with the reported role of the BCR/ABL-regulated hnRNP A1, K and E2 as a positive regulator of BCR/ABL stability through the SET-dependent inhibition of PP2A, a direct enhancer of Myc translation, and as an inhibitor of C/EBPa-dependent myeloid maturation of blast crisis CML progenitors, respectively.

3.6. **[192] In Chronic Myeloid Leukemia Cytotoxic T Cell Responses to BMI-1 Protein Correlate with Higher Expression of *BMI-1* in Leukemia Progenitors, and May Contribute to Improved Outcome After Allogeneic Stem Cell Transplantation.** *Yong*. The polycomb group (PcG) proteins BMI-1 and EZH2 are key regulators of self-renewal processes in normal and leukemic stem cells. Of these two PcG proteins, BMI-1 is more highly expressed in chronic myeloid leukemia (CML) than in normal stem cells, and is associated with a more rapid disease progression in patients who are treated with drug therapy alone, implying that an increased level of "stem-ness" conferred by BMI-1 contributes to leukemogenicity. Conversely, CML patients with high BMI-1 expression prior to allogeneic stem cell transplantation (SCT) have better overall survival post-transplant (Mohty, et al, Blood 2008). To investigate the potential of PcG proteins as leukemia-associated antigens, and targets for graft-versus-leukemia (GVL) effects, we studied a cohort of 86 CML patients (54 chronic phase, 32 advanced phase) who received T-cell depleted SCT with T-cell add-back on day 45-100 post-SCT from HLA-identical sibling donors. Using quantitative real-time PCR, we measured the expression of *EZH2, BMI-1* and its target for repression, *CDKN2A* (encoding p16INK4A) in CD34+ progenitors and their CD34-negative counterparts. Using flowcytometric detection of intracellular cytokines IFN- or TNF- , and degranulation marker CD107a, in CD8+ cytotoxic T lymphocytes (CTL), we assessed immune responses to BMI-1 (TLQDIVYKL and CLPSPSTPV) and EZH2 (YMCSFLFNL and SQADALKYV) peptides in 25 HLA-A*0201+ patient-donor pairs. Seven of 17 (41%) HLA-A*0201+ CML patients had native immune responses to BMI-1 peptide, which was associated with higher *BMI-1* expression in CD34+ progenitors (p=0.04, Mann-Whitney *U* test). Five of 25 (20%) healthy HLA-A*0201+ sibling donors had detectable immune responses to BMI-1 peptide. EZH2 was less immunogenic compared to BMI-1 in both patients and donors. The majority of peptide-specific CTLs

analyzed by peptide-specific dextramers had central memory phenotype. BMI-1- or EZH2-specific T cells were readily detected after 7-day cultures using an ELISPOT assay in 75% of donors or patients where peptide-specific CTLs were detected ex-vivo. A higher expression of *BMI-1* in CML patients pre-SCT and correspondingly lower expression of its target for repression, *CDKN2A,* was associated with improved leukemia-free survival (p=0.01), and reduced disease-related death (p=0.0001). In four HLA-A*0201+ patients whose donors had immune responses to PcG peptides, BMI-1 or EZH2-specific T cell responses were detected in the first 120 days post-SCT. CML patients who had donors with immune responses to BMI-1 peptide had improved leukemia-free survival compared to patients whose donors were non-responders (80% vs. 60% respectively). Immune responses to PcG proteins, in particular to BMI-1, may be relevant for disease control by GVL effects. Unlike CTLs specific for primary granular proteins such as proteinase 3 and elastase, which are also highly expressed in CML cells, CTLs against BMI-1 and EZH2 may be less susceptible to selective deletion processes resulting in tolerance, as these proteins are less ubiquitously expressed by mature progenitors which are expanded in CML, and are therefore good GVL and immunotherapy target candidates in CML. Furthermore, these CTLs have the potential to target leukemia stem cells.

4 Initial Therapy [337-342]

4.1. **[337] 24 Months Update of the TOPS Study: a Phase III, Randomized, Open-Label Study of 400mg/d (SD-IM) Versus 800mg/d (HD-IM) of Imatinib Mesylate (IM) in Patients (Pts) with Newly Diagnosed, Previously Untreated Chronic Myeloid Leukemia in Chronic Phase (CML-CP).** *Baccarani*. Background: 12-month results from TOPS showed an advantage for the high dose (800mg/day, HD-IM) arm for: (1) time to achievement of major molecular response (MMR); MMR rates at 3 and 6 months (mos); and (2) complete cytogenetic response (CCyR) at 6 mos (Guilhot et al. [TOPS] ASH 2008). There was no significant difference in the MMR rates at 12 mos, the primary endpoint, and results support 400 mg/day as the standard initial dose for imatinib (SD-IM) in CML-CP pts. The purpose of this update is to assess the outcome of the two arms at 24 mos, as well as the impact of dose intensity (DI) and dose interruption on pts outcome. Methods: 476 pts with newly diagnosed CML-CP were randomized 2:1 to receive HD-IM (n = 319) or SD-IM (n = 157) at 103 sites in 19 countries. The endpoints assessed at 24 mos were: rates of CCyR and MMR, event-free (EFS), progression-free (PFS), and overall survival (OS). CCyR and MMR rates were calculated based on pts with available assessments at specified timepoints. Adverse events (AE) and laboratory results were also monitored. Additional analyses were performed using DI (< 600 mg/d and ≥ 600 mg/d) and number of dose interruptions (periods of zero dose of > 5 days) as variables. In the trial dose interruptions were specified as an AE management strategy prior to dose reduction. Results: At 24 mos, 129/157 (82%) of SD-IM and 247/319 (77%) of HD-IM pts remained on study treatment. There was no significant difference in rates of MMR at 12 mos (SD-IM: 40% vs. HD-IM: 46%), 18 mos (52% vs 47%) or 24 mos (54% vs 51%) (intention to treat analysis). There was no significant difference in the cumulative rates of CCyR at 24 mos (76% in each arm). Overall, 9 (6%) pts on SD-IM and 15 (5%) pts on HD-IM had experienced an event (loss of complete hematologic response, loss of major cytogenetic response, progression to accelerated phase and blast crisis, or death due to any cause). There were no significant differences in estimated EFS (SD-IM: 95% vs. HD-IM: 95%), PFS (97% vs. 98%), or OS (97% vs. 98%) at 24 mos. The most common grade 3/4 hematologic AEs were neutropenia (17.8% vs. 28.2%) and thrombocytopenia (8.9% vs. 19.9%), and for the most part occurred during the first 12 mos. The most common grade 3/4 nonhematologic toxicities for SD-IM and HD-IM were rash (2.5% vs 5.7%), diarrhea (1.3% vs 6.0%) and myalgia (0.6% vs 3.5%), respectively. The most common reasons for discontinuation in both arms were AEs (4.5% vs. 10.7%) and unsatisfactory therapeutic effect (7.0% vs 7.2%). Median DI was 400 mg/d in the SD-IM arm and 728 mg/d in the HD-IM arm. Dose interruptions for > 5 days occurred more frequently in the HD-IM arm (71% vs. 44%). Pts in both arms combined who had ≤ 1 dose interruption during the first 12 mos achieved higher MMR rates at 12 (57.1% vs 33.3% for ≤ 1 vs > 1 interruption; P < 0.0001) and 18 mos (72.6 vs. 46.8; P < 0.0001), faster time to MMR (P = 0.0002), and higher CCyR rates at 12 mos (88.8 vs 63.8; P < 0.0001), compared with pts who had > 1 dose interruption during the same period. On the SD-IM arm pts with ≤ 1 (vs > 1) dose interruption also had higher MMR rates at 12 and 18 mos (12 mos: 49.6% vs 22.2%, P = 0.04; 18 mos: 70.9% vs 50%, P = NS). Comparing pts in the HD-IM arm with DI ≥ 600mg/d for the first 12 mos vs DI < 600 mg/d, the MMR rates at 12 mos (62.4% vs 34.1%, P < 0.0001) and 18 mos (75.2% vs 40.3%, P < 0.0001) were higher, the time to MMR was faster (P < 0.0001), duration of MMR was longer (P = 0.0141) and CCyR rates at 12 mos (89.6% vs 70.3%, P < 0.0001) were higher for pts with DI ≥ 600mg/d. Conclusions: TOPS continues to confirm the safety and efficacy of 400 mg/day IM for newly diagnosed pts with CML-CP, with very similar results to IRIS. HD-IM was also safe and generally well tolerated, but overall did not result in better outcomes at milestones up to 24 months. Frequent or prolonged dosage interruptions on IM adversely affect patient outcomes and should be avoided. These data emphasize the importance of maintaining dose intensity in CML-CP pts treated with imatinib. The TOPS study will continue to assess the impact of dose intensity on long-term outcomes.

NOTES ON PRESENTATION

2 to 1 allocation 319 vs 157. Primary endpoint was MMR at 12 months. Recently published in JCO. 24 MONTH DATA. There were significant differences early on but these disappear at 12 and certainly at 24 months. Patients with 2 or more dose interruptions had lower rates of MMR and CCR.

TOPS @ 24 months	**Overall**		**400mg**		**800 mg**		**Comment**
Parameter	No.	%	No.	%	No.	%	
No. patients total			157		319		
CCyR				76		76	

MMR				54		51	
¾ neutropenia				18		28	
¾ thrombopenia				9		20	
OS							NS

4.2. **[338] Efficacy of Dasatinib in Patients (pts) with Previously Untreated Chronic Myelogenous Leukemia (CML) in Early Chronic Phase (CML-CP).** *Cortes.* Dasatinib, a potent inhibitor of ABL and SRC, is approximately 300 times more potent than imatinib in vitro and has significant activity in pts with CML-CP resistant or intolerant of imatinib (IM). We initiated a phase II trial to study efficacy and safety of dasatinib in pts with previously untreated CML-CP. Aims: To investigate the efficacy and safety of dasatinib as initial therapy for patients with CML-CP. Methods: The primary objective was to estimate the proportion of pts attaining major molecular response at 12 months (mo). Pts with previously untreated CML-CP within 6 months from diagnosis were eligible and received dasatinib 100 mg/day, randomized to either 50 mg-twice-daily (BID) or a 100 mg-once-daily (QD). Results: Sixty-two pts have been enrolled (31 on the QD schedule, 31 BID). Median age was 47 years (yrs) (range 18–76 yrs). Median follow-up is 24 months (mo) (range, 1 to 39 mo). All 45 pts who were not in CHR at the start of therapy achieved CHR. Among 50 pts followed for at least 3 months, 49 (98%) achieved complete cytogenetic response (CCyR). Major molecular response has been achieved in 35 (70%), including 5 (10%) with complete molecular response. The CCyR rate at different timepoints (intention-to-treat) compares favorably to that observed in historical controls treated with imatinib 400mg or 800 mg daily. Major molecular response was achieved by 45% by 12 mo and 71% by 24 mo (corresponding rates with imatinib 400mg 34% and 55%, and with imatinib 800mg 58% and 66%, respectively). There was a trend for higher MMR rate with the QD schedule: overall 75% vs 65% (p=0.54), and by 12 months 52% and 38% (p=0.54). Grade 3-4 non-hematologic toxicity (regardless of causality) included fatigue (6%), pain (muscle or joint) (6%), dyspnea, neuropathy and memory impairment (5% each). Pleural effusion occurred in 13% evaluable pts (grade 3-4 in 2%). Grade 3-4 hematologic toxicity (transient) included thrombocytopenia 10%, neutropenia 21%, and anemia 6%. Thirty (48%) of 62 pts required transient treatment interruptions. The actual median daily dose for all pts was 100mg. There is no significant difference in grade 3-4 toxicity by treatment schedule but there was a trend for less pleural effusion with QD (3%) vs BID (10%; p=0.26). Three pts lost CCyR: 2 because of non-compliance, 1 due to treatment interruption because of pleural effusion. 24 month EFS (event= loss of CHR, loss of MCyR, AP/BP, death, or off because of toxicity) is 88%. All patients are alive. Conclusion: Rapid CCyR occurs in nearly all patients with previously untreated CML-CP treated with frontline dasatinib therapy; the MMR rate at 18 months was 71%, with a favorable toxicity profile. Because of favorable trends in response and toxicity, only QD arm will continue accrual.

Mo on therapy	Percent with CCyR		
	Dasatinib	Imatinib 400mg	Imatinib 800mg
3	82	37	63
6	94	54	85
12	98	65	89
18	89	67	89
24	84	67	88
30	83	67	89

NOTES ON PRESENTATION

Single arm, single centre study.

Primary endpoint was MMR at 12 months. 72 patients on study median FU 2 years. 71 eval?? Grade ¾ M/S pain in 14%. Fatigue in 11%. 2 patients with grade 3 pleural effusions. 53% patients required dome form of dose interruption. By 36 months less than 50% patients stayed on 100mf full dose.

Dasatinib up front	**Dasat**		**Imat 400mg**		**Imat 800 mg**		**Comment**
Parameter	No.	%	No.	%	No.	%	Imat NOT randomized – historical controls.
No. patients total			157		319		
No. patients analysed	70						
Median age							
Prior mutations							
Median previous disease duration							
Median follow up (range)							
Median dose delivered							
CHR							

MCyR							
CCyR	67	96					
MMR		89					At 24 months
CMR		8					
Median survival							
¾ anaemia							
¾ neutropenia		25					
¾ thrombopenia		24					
Lipase							
ALT							
Pleural effusion ¾							
QTc prolongation							
Rash ¾							
Diarrhoea ¾							
Discontinued							
OS							
EFS		90					At 3 years

58 74 34 MMR d i4 i8 at 12 months. Not sure got this right…

4.3. **[339] Randomized Comparison of Imatinib 800 Mg Vs. Imatinib 400 Mg +/- IFN in Newly Diagnosed BCR/ABL Positive Chronic Phase CML: Analysis of Molecular Remission at 12 Months; The German CML-Study IV.** *Hehlmann*.
Initial reports that high dose imatinib results in better responses more rapidly than standard dose imatinib remain controversial. The German CML Study Group therefore compared imatinib 800 mg (IM 800) with standard dose imatinib +/- IFN (IM 400, IM 400 + IFN) in newly diagnosed, not pretreated CML with regard to molecular response at 12 months and survival in a randomized clinical trial. By April 30, 2009, 1026 chronic phase CML patients have been randomized (326 for IM 400, 338 for IM 800, 351 for imatinib + IFN). Comparison was for molecular and cytogenetic remissions, overall (OS) and progression free (PFS) survival and toxicity. 1015 patients were evaluable at baseline, 904 for survival analysis (294 for IM 400, 286 for IM 800, 324 for IM 400+IFN), 790 for cytogenetic (analysis of at least 20 metaphases required) and 823 for molecular response. The three treatment groups were similar regarding median age, sex, median values of Hb, WBC, platelets and distribution according to the EURO score. Median follow-up was 25 months in the imatinib 800 mg arm and 42 months in the imatinib 400 mg +/-IFN arms. The difference is due to the fact that at first the IM 800 arm was designed for high risk patients only and opened up to all risk groups in July 2005. The median daily doses of imatinib were 626 mg (209-800 mg) in the IM 800 arm and 400 mg (184- 720 mg) in the IM 400 +/- IFN arms. Of 218 patients receiving imatinib 800 mg and evaluable for dosage at 12 months, 100 (45.9%) received more than 700 mg/day, 27 (12.4%) 601-700 mg, 37 (17.0%) 501-600 mg, 48 (22.0%) 401-500 mg and only 6 (2.8%) 400 mg/day or less. The cumulative incidences at 12 months of complete cytogenetic remission (CCR) were 52.3%, 64.9% and 50.6%, and of major molecular remission (MMR) 30.2%, 54.3% and 34.6% with IM 400, IM 800 and IM 400 +IFN, respectively. The cumulative incidences of achieving CCR and MMR with IM 400, IM 800 and IM 400+IFN at 6, 12, 18 and 24 months after start of treatment are summarized in the table. MMR at 12 months was reached faster with IM 800 than with IM 400 (p=0.0003) or IM400+IFN (p=0.0131). Optimal molecular response (OMR=<0.01% BCR-ABL according to the international scale) was reached with IM 800 after a median of 31.3 months vs. 47.5 and 42.5 months with IM 400 +/- IFN. Also CCR was reached faster with IM 800 (p<0.01). The more rapid achievement of MMR with IM 800 was observed in low and intermediate risk patients with little or no difference in high risk patients. In an analysis "as treated" patients receiving more than 600 mg/day reached remissions faster than those receiving lower dosages (CCR after a median of 7.8 vs. 8.9 months, MMR after a median of 10.4 vs. 12.9 months). At the time of this evaluation, OS (92% at 5 years) and PFS (88% at 5 years) showed no difference. Type and severity of adverse events (AE) at 12 months did not differ from those expected (all grades and grades III/IV). Hematologic (thrombocytopenia 7% vs. 4%) and non-hematologic AEs (gastrointestinal 35% vs. 15-24% and edema 29% vs. 16-19%) were more frequent with IM 800, fatigue (14% vs. 7-13%) and neurological problems (15% vs. 6-7%) more frequent with IM 400 + IFN (all grades). These data show a significantly faster achievement of MMR at 12 months with IM 800 as compared to IM 400 +/- IFN. So far, this faster response rate did not translate into better OS or PFS. Hence IM 400 should still be considered as standard of care. With some individual dose adjustments tolerability of IM 800 was good. Longer observation is required to determine whether this more rapid achievement of MMR and CCR will have a long term impact or not.

Time after start of treatment	Cumulative incidences of achieving a							
	CCR (%)				MMR (%)			
	IM400 n=259	*D*	IM800 n=241	IM400+IFN n=290	IM400 n=267	*D*	IM800 n=261	IM400+IFN n=295
6 mo	22.7	*12.4*	35.1	18.4	6.4	*11.8*	18.2	6.2
12 mo	52.3	*12.6*	64.9	50.6	29.0	*23.1*	52.1	34.3
18 mo	69.3	*6.7*	76	70.8	48.1	*18.9*	67.0	55.4
24 mo	78.3	*6.3*	84.6	77.6	63.3	*14.1*	77.4	63.8

NOTES ON PRESENTATION

Primary EP is MMR
1022 randomised. 1016 evaluable.
Median obs from 28-47 m.
882 for cyto
Neuro and fatigue worse in IFN. No diff if only ¾ considered.
MMR reached quicker with 800 but cumulative total is the same.
MMR rate at 12 month was higher with 800 (NB cf TOPS!!!) IFN did not convey any advantage.
OS by therapy at 5 years NS. Combo and HD arm
MMR rate with 8 is higher
Time to MMR is shorter
Diff in low and inter, not high
5yr OS 92 PFS 89. No diff between treatment groups.
'Optimal' dose higher than 400????
cfTOPS??

4.4. **[340] Significant Higher Rates of Undetectable Molecular Residual Disease and Molecular Responses with Pegylated Form of Interferon a2a in Combination with Imatinib (IM) for the Treatment of Newly Diagnosed Chronic Phase (CP) Chronic Myeloid Leukaemia (CML) Patients (pts): Confirmatory Results at 18 Months of Part 1 of the Spirit Phase III Randomized Trial of the French CML Group (FI LMC).** *Guilhot*. Background: IM 400 mg daily is the front-line treatment of CP CML, but provides only 50% major molecular responses (MMR) at 18 months (Mo). Aims: we designed a phase III randomized multicenter open-label prospective trial comparing IM 400 mg/d (n=159) with 3 experimental arms: IM 600 mg/d (n=160), IM 400 mg/d combined to s/c cytarabine (Ara-C), (20 mg/m^2/d, d15-28 of 28-day cycles)(n=158) and IM 400 mg/d combined to s/c Peg-IFN2a (90 µg/wk) (n=159). Methods: Pts were allocated at a 1.1.1.1 ratio, stratified by Sokal risk groups. Molecular assessments were centralized, blinded and calculated according to the international standardized scale (IS). The purpose of this trial was to first determine whether higher doses of IM or combining IM with interferon or Ara-C would result in higher rates of molecular responses and if so, in better survival. Thus the trial was designed to be conducted according to 2 parts. During the part 1, the increased dose of IM or a combination regimen would be considered as promising at 1 year, if it increased the 4 log reduction response rate by at least 20 percentage points, e.g. from 15% to 35%, with an acceptable tolerability A planned interim analysis of 636 pts based on an optimal molecular response (OMR = BCR-ABL/ABL ratio ≤ 0.01) (=0.85%, =10%) at 1 year has suggested the superiority of the combination of Peg-IFN2a and imatinib (ASH 2008). We now report the 18 months update of this planned interim analysis of part 1 of the trial. Results: Pts of the part 1 were recruited between 9/2003 and 10/2007, median age 51 yrs (18-82), 62% males; Sokal score was low 37%, intermediate 39% and high risk 24%. Median follow-up was 42 Mo. (range 18-73) for alive patients. MMR, OMR and undectable molecular residual disease (UMRD) rates are described in Table 1. During the first year of treatment the median dose of IM was 400 mg for the 3 arms including IM 400 and 590 mg for IM 600; the median dose for Peg IFN2a was 54 µg per week (range11-166) and was 24mg per day (range 10-40) for Ara-C. Overall, 45% of the pts discontinued Peg-IFN2a during the first 12 months. Of interest, duration of treatment with Peg-IFN2a had an impact on responses. In pts who have been treated less than 4 months as compare to more than 12 months, rate of MMR, OMR and UMRD increased from 48% to 82%, 23% to 49% and 8% to 20% respectively. Grade 3/4 neutropenia and/or thrombocytopenia occurred during the first year in 8% IM-400, in 14% IM-600, in 41% IM-Ara-C and in 40% IM-PegIFN arms respectively. No significant infection rates were observed between the 4 arms. Grade 3/4 non-haematological toxicities occurred in 19% IM-400 (oedemas, muscle cramps), in 30% IM-600, in 27% IM-Ara-C (diarrhoea) and in 31% IM-PegIFN pts (skin rashes, asthenia). Conclusions: Based on these results and as recommended by the Independent Data and Ethics Monitoring Board, the CML French Group (FI-LMC) stopped accrual into the IM 600mg and IM 400mg + Ara-C arms and is currently continuing with IM 400 mg as control arm and the combination IM400mg + Peg-IFN2a as best experimental arm. This second part of the trial aims to confirm if achieving significant higher molecular responses will translate into a better event free and overall survival.

Rates at 18 months (ITT)	IM+Peg-IFN2a	IM 400 mg	IM 600 mg	IM + Ara-C	P value. PegIFN versus IM 400mg
MMR	62%	41%	52%	53%	p = .0001
OMR	36%	19%	25%	19%	p = .0007
UMRD	15%	4%	7%	5%	p = .0013

NOTES ON PRESENTATION

695 patients
44 months median follow up
4 groups. 4, 6, ara-C, IFN
No difference in survival
MMR at 24

71, 63, 62, 48
IF, 6, 4, A
Undetectable
I, 6, 4, A
22, 11 11 10
45% disc. PEG
Relation between duration of PEG and response
42% of patients off IFN at 12 months

French SPIRIT	**Imat4+IFN**		**Imat 400mg**		**Imat 800 mg**		**Comment**
Parameter	No.	%	No.	%	No.	%	Imat NOT randomized – historical controls.
No. patients total							
No. patients analysed							
Median age							
Prior mutations							
Median previous disease duration							
Median follow up (range)							
Median dose delivered							
CHR							
MCyR							
CCyR							
MMR							
Median survival							
¾ anaemia							
¾ neutropenia							
¾ thrombopenia							
Lipase							
ALT							
Pleural effusion ¾							
QTc prolongation							
Rash ¾							
Diarrhoea ¾							
Discontinued							
OS							
EFS							

4.5. **[341] Efficacy of Nilotinib in Patients (Pts) with Newly Diagnosed, Previously Untreated Philadelphia Chromosome (Ph)-Positive Chronic Myelogenous Leukemia in Early Chronic Phase (CML-CP)** *Cortes.* Background: Nilotinib, an oral tyrosine kinase inhibitor with increased selectivity against Bcr-Abl and approximately 30-fold more potent than imatinib, is effective in CML after imatinib failure. We initiated a phase II study to evaluate the efficacy of nilotinib as 1st line therapy in pts with newly diagnosed CML-CP. Aims: To investigate the efficacy and safety of nilotinib as first-line therapy for pts with CML-CP. Methods: The primary objective was to estimate the proportion of pts attaining major molecular response (MMR) at 12 months (mo). Pts with untreated CML-CP within 6 mo from diagnosis were eligible and received nilotinib 400 mg twice daily. A cohort of patients with previously untreated CML in accelerated phase (AP) was also included. Results: Sixty-five pts (61 CP, 4 AP) have been treated for a median of 17 mo (range 1 to 43). The median age was 46 years (range 19 to 86). Among 48 pts who were not in CHR at the start, 47 (98%) achieved CHR (one discontinued after 2 weeks without adverse events). Among 51 pts followed for at least 3 mo, 50 (98%) achieved a complete cytogenetic response (CCyR). MMR has been achieved in 32 (63%) pts, including 12 (24%) with a complete molecular response. The rate of CCyR at different time points (intention-to-treat) for pts in CP compares favorably to that observed in historical controls treated with imatinib 400 mg or 800 mg daily. MMR was achieved by 55% at 12 mo and 53% at 24 mo (corresponding rates with imatinib 400 mg 34 and 55%, and with imatinib 800 mg 58% and 66%, respectively). Grade 3-4 hematologic toxicity (transient) included thrombocytopenia 11%, neutropenia 12%, and anemia 5%. Grade 3-4 non-hematologic adverse events (regardless of causality) included elevation of bilirubin in 8% and lipase in 6%, and non-neutropenic fever in 6%. 24 (37%) pts had transient treatment interruptions and 11 (17%) had dose reductions. The actual median dose is 800 mg daily. Ten pts have discontinued therapy: 4 pts for toxicity, 2 because of transformation to accelerated or blast phase, and 4 for other reasons. 24 mo EFS (event= loss of CHR, loss of MCyR, AP/BP, death, or off because of toxicity) is 90%. All patients are alive. Among pts in AP, 3 achieved CCyR (all of them sustained); one patient progressed to blast phase and died. Conclusion: Nilotinib 400 mg twice daily induces a CCyR in nearly all patients as early as 3 mo after the start of therapy and MMR in more than 50% at 12 months with a favorable toxicity profile.

Months on therapy	Percent with CCyR		
	Nilotinib	Imatinib 400mg	Imatinib 800mg
3	90	37	63
6	96	54	85
12	97	65	89
18	93	67	89
24	93	67	88
30	92	67	89

NOTES ON PRESENTATION

Starting 400 twice daily.
74, 67 CP patients
58 made it to 3 months cor CCR
83% MMR at 24 months with 21% CMR
24 mos, 93% CCR
92% EFS. 1 patient has transformed.
Non-haem gd 3-4, bilirubin and lipase
Haem
¾ N = 11, plt 13%
43% patients required treatment interruptions.

4.6. **[342] Chronic Myeloid Leukemia in Pediatrics – First Results From Study CML-PAED II.** *Suttorp*. Background: Chronic myeloid leukemia (CML) is a rare malignancy in pediatrics. In this decade -like in adults- imatinib meyslate (IMA) has been established also as first line treatment for children with CML while allogeneic stem cell transplantation (SCT) as treatment option is postponed for those cases becoming intolerant or refractory to tyrosine kinase inhibitor (TKI) treatment. However, results from controlled trials in children are lacking so far. We here report an analysis of pediatric data from patients (pts) with newly diagnosed Philadelphia-chromosome positive (Ph+) CML on up-front treatment with IMA. Pts and Methods: According to protocol CML-PAED II pediatric pts with confirmed diagnosis of Ph+ CML were treated in CP with IMA 300 mg/sqm once daily, while in accelerated phase (AP) or in blastic phase (BC) the dose was increased to 400 mg/sqm and 500 mg/sqm (bis daily), respectively. Initial and long-term clinical and laboratory data, treatment response and side effects were reported to the study center on standardized forms by the treating physician. Specimen from peripheral blood (pB) and bone marrow (BM) were assessed by cytogenetics and by quantitative RT-PCR for BCR-ABL transcript rates in central laboratories for standardized monitoring in three months intervals. Results: From 1. Jan 2004 until 31. Mrch 2009 a total of 51 pts (21 female, 30 male; median age: 10.6 yrs [range:1-20 yrs]) were registered: 10 pts with ongoing IMA treatment were recruited and analyzed retrospectively while 41 pts were enrolled prospectively from centers in Austria (n=1), Czech Rep. (n=6), Germany (n=40), Italy (n=1), Netherlands (n=1), Slovak Rep. (n=2). Stages of disease were: CP n=47; AP n=1; BC n=3 (two myeloid). Those four pts diagnosed in AP and BC underwent early SCT. Observed side effects in the whole group included: nausea (n=9), muscle pain (n=7), edema (n=3), rhabdomyolysis (n=1, short interruption of IMA), reduced blood cell count (n=2, short interruption of IMA in one pt), biochemical alterations in bone metabolism [for details see: N Engl J Med 2006;354:2006] (n = 8), impaired longitudinal growth (n=1, [Haematologica 2009;94:1177]). Two pts experienced intolerance (muscle pain) or toxicity (hepatic), respectively, therefore stopped IMA and were put on dasatinib after 4 and 10 months, respectively. Having achieved complete cytogenetic response (CyR) and 2 log-fold reduction of BCR-ABL transcript rate, one pt opted for SCT from her HLA-identical brother after 15 mo of treatment. Response rates in advanced stages of CML were as follows: in BC (n=3) two pts became hematological responders (HR), one pt exhibited partial HR. The only one pt diagnosed in AC exhibited partial CyR but complete HR. A landmark analysis in pts entering CML-paed II in CP exhibited that 2/42 pts (5%) had no complete HR at month 3; 2/28 (7%) had no complete CyR at month 12, and 2/19 (15%) pts achieved no major molecular response (MMR, defined as >0.1% BCR-ABL [Blood 2006;108:28–37]) at month 18 after start of IMA. Each two of those four patients with incomplete response (one pt with no CyR at month 12, one pt with no MMR at month 18) underwent SCT from a sibling donor and the other two pts stopped IMA and were put on dasatinib. With a median follow-up of 19 months (range: 0-63 months) all 47 pts diagnosed in CP are alive. Of note none of the six pts (median age at diagnosis: 5 yrs; range 1–13 years) treated by imatinib meanwhile for >36 months have opted for SCT. Conclusion: Keeping in mind that the number of pediatric pts is still small, IMA treatment for children and adolescents with CML in CP is associated -like in adults- with high treatment response rates. Refractoriness to IMA is uncommon and side effects seem tolerable, as only 10% of the total cohort stopped imatinib and were put on 2nd generation TKI. However, disturbances of bone metabolism and longitudinal growth impairment may be of special concern in this not yet outgrown cohort [N Engl J Med 2006;354:2006, Blood 2008;111:2538; Haematologica 2008;93:1101; Lancet 2008;372:111; Int J Hematol; 2009;89:251; Haematologica 2009;94:1177]. Only 3/47 pts not diagnosed in advanced phases of CML so far underwent SCT thus underlining that also in pediatrics SCT has been shifted to a second-line strategy for high-risk patients and those who failed therapy with IMA.

5 Prognostic Factors [505-510]

5.1. **[505] Sustained Complete Molecular Response to Imatinib in Chronic Myeloid Leukemia (CML): a Target**

Worth Aiming and Achieving? *Verma*. Background: Therapy with imatinib and other tyrosine kinase inhibitors leads to complete cytogenetic response (CCyR) in 80-90% of patients in chronic phase (CP) of CML, but most patients have residual disease documented by real-time quantitative polymerase chain reaction (PCR). Only a minority of patients achieve complete molecular response (CMR), as defined by undetectable levels of BCR-ABL fusion transcripts by PCR with sensitivity of at least 4.5 logs. Achieving CMR may offer the possibility of treatment discontinuation. Aims: To identify patients with sustained CMR (CMR of at least 6 months consecutively on 2 different dates) so as to define i) incidence of sustained CMR, ii) significance in long-term outcome (event-free survival, survival, transformation), and iii) predictive factors for CMR. Methods: We analyzed records of all patients with CML in early chronic phase (ie, within 12 months from diagnosis) treated with imatinib as frontline therapy at MD Anderson Cancer Center from July 2000 to Aug 2009. Major molecular response was defined as a BCR-ABL/ABL ratio of ≤0.05%, and CMR as undetectable transcripts in an assay with a sensitivity of at least 4.5 logs. Molecular responses were considered sustained only if they met the criteria for response in at least 2 consecutive assays separated over a period of at least 6 months. All patients were followed by PCR every 3 months for the first 1-2 years, then every 3-6 months. Rates of molecular response are reported on an intention-to-treat analysis. Results: 281 patients were included: 271 in CP and 10 in CP with clonal evolution at the time of diagnosis. The median age was 48 years (range 15-83), 119 (42%) were females, median CML duration 1 month (mo) (range 0-12). Seventy-three (26%) patients received an initial imatinib dose of 400 mg and 208 (74%) with 800 mg. The median follow-up is 65 mo (range 2-107) with 249 (89%) treated for over 12 mo, 225 (80%) for over 24 mo, 211 (75%) for over 36 mo, 154 (55%) for over 60 months, and 29 (10%) treated for over 96 mo. 55 (20%) have discontinued therapy (34 -12%-, because of resistance, and 21 -7%- because of intolerance). Overall, 248 (88%) achieved a CCyR, 80 (28%) a MMR without CMR, and 123 (44%) a CMR in at least one measurement. MMR was sustained in 95 (34%) and CMR in 84 (30%). The median time to CCyR was 3 mo (range 2-30), to sustained MMR 18 mo (range 6-78), and to sustained CMR 30 mo (range 6-84). The median event free survival was not reached for patients in CCyR with CMR/MMR without CMR/no MMR. Among patients who did achieve a CCyR, those that had a sustained CMR by 24 mo of therapy had an EFS of 100% at 5 yrs, compared to 96% for those with MMR but no CMR, and 86% for those with CCyR but no MMR (p=0.02). The rate of survival free from transformation to accelerated or blast phase at 5 yrs was 100% for those with CMR at 24 mo, compared to 96% for those with MMR but no CMR, and 91% for those with CCyR but no MMR (p=0.1). On univariate analysis, factors predicting sustained CMR were platelet count >450x109/L (p=0.001), CCyR at 3 mo (p=0.0005) and at 6 mo (p<0.0001). Conclusion: These results suggest that achieving a CMR is an important endpoint for patients with CML treated with imatinib as initial therapy. Treatment strategies that may increase the rate of sustained CMR should be investigated.

5.2. **[506] Bcr-Abl Kinetics Suggest Self-Renewing Leukemic Cells Are Reduced During Imatinib Treatment.** *Stein*. Background: In a majority of chronic myeloid leukemia patients treatment with imatinib (IM) induces durable hematologic, cytogenetic, and molecular responses, which in turn results in a dramatic improvement of progression-free survival. A new challenge for further optimizing the management of CML is to determine if treatment with TKIs such as IM could eventually cure the patients, particularly those with sustained undetectable disease, by reducing or eliminating the leukemic self-renewing cell (LSC) population thought to drive the disease. Given the challenges in directly assessing LSC burden in patients, we have applied mathematical modeling to molecular response data from patients on IM therapy to infer whether LSC levels can be reduced during IM treatment. Methods: To conduct our study we have utilized 281 patients on the IM arm of the International Randomized Study of Interferon Versus STI571 (IRIS) trial (Drucker et al., NEJM, 2006; 355, 2408). The 281 patients maintained a 90% dose intensity over the course of treatment (up to 7 years) and had a sufficient number of PCR samples above the quantitation limit to support parameter estimation. We have modeled the Bcr-Abl/Bcr transcript ratio time course R(t) as a biexponential (R(t) = Ae t + Be t) and estimated the model parameters for each patient using maximum likelihood methods. The parameter < 0 describes the rapid initial decline in log10(R) upon treatment start while describes the shallower slope of the subsequent log10(R) kinetics (figure 1a). Results: nearly every patient response trajectory (93%-263/281) was well-described by the bi-exponential model. The key parameter , corresponding to the steady-state per-year reduction in log10 transcript levels, ranged from a minimum = -9.2, lower quartile = -1.1, median = -0.6, upper quartile = -0.2, maximum = 10.5 (see figure 1b). Approximately 21% (54/263) of patients had >0, suggestive of molecular relapse, while 79% (209/263) have <0, corresponding to continued transcript decline. Discussion: The durable reduction in Bcr-Abl transcripts in the majority of patients has two possible interpretations: either LSCs are depleted at a rate , or LSCs are not depleted but leukemic progenitor cells in the bone marrow have a median half-life of over one year in many patients. However, leukemic progenitor dynamics appear to occur at a time scale of months (Abe et al, Int J Hematol. 2008; 88, 471); thus a durable reduction in transcript level must correspond to a depletion of LSCs. Our hypothesis that IM induces LSC depletion is also consistent with the observation that 50% of patients maintain undetectable Bcr-Abl transcript levels upon treatment discontinuation (Guastafierro et al, Leukemia Res. 2009; 33, 1079). Conclusions: Mathematical modeling which combines pathophysiologic principles of CML with 7-year molecular response data in individual patients predicts that most patients (~79%) experience reduction in LSC burden while on sustained IM treatment. In the future, this modeling approach can be used to analyze data from patients who stop IM after achieving CMR in the context of carefully conducted clinical trials, with the goal of assessing (a) the potential impact of IM interruption on LSC levels, particularly in those patients who relapse upon stopping IM and then are re-induced, and (b) whether the probability of achieving cure depends on duration of CMR, the steepness of response prior to achieving CMR ( ), and/or additional factors.

5.3. **[507] The Functional Activity of the OCT-1 Protein Is Predictive of Molecular Response and Survival in CP-CML Patients Treated with Imatinib: A 5 Year Update of the TIDEL Trial.** *White*. The major active influx protein for imatinib into target BCR-ABL positive cells is the organic cation transporter OCT-1. We have previously demonstrated that the functional activity of the OCT-1 protein (OCT-1 activity) is predictive of molecular response in TIDEL (trial of imatinib 600 mg/day with selective dose intensification in untreated CP-CML) The OCT-1 activity (OA) is measured in mononuclear cells from untreated CML patients by calculating the intracellular concentration of 14-C imatinib less the intracellular concentration in the presence of OCT-1 inhibition. To address the question of whether OA is predicting only the rate of response, we now

investigate the impact of OA on response and progression at 5 years. There is a significant difference in the achievement of MMR (p=0.007) and CMR by 60 months (p=0.032) (Table 1). Six patients developed kinase domain mutations over the course of this study. 5/6 had low OA. Significantly, for the first time addressing Event Free Survival (events defined as loss of CHR, MCR or CCR, progression to AP or BC or change of therapy due to unsatisfactory efficacy), we demonstrate that more patients with high OA are event free at 5 years when compared to patients with low OA (Table 1). To determine whether the detrimental effect of low OA on survival was more significant in those patients with OA in the lowest quartile (Q1) we compared the response of Q1 patients to all other patients (Table 2). These data demonstrate importantly, that patients in Q1 have significantly poorer outcomes, than the remainder of the patient cohort. In previous analyses we have shown that the effects of a low OA can be partially overcome by higher imatinib doses. Limiting the analyses to those patients receiving <600mg average daily dose over the first 12 months there was a significant difference in the achievement of MMR (low OA (n=11) 27%: high OA (n=12) 92% p=0.021) and EFS (36% vs 75% p=0.03). In patients receiving ≥600 mg there was no significant difference between the groups, reinforcing the importance of dose. In 45 patients we examined the expression of OCT-1 mRNA for prediction of MMR, CMR, EFS and mutation development. Dividing the patients into low and high OCT-1 expression about the median we found that the level of mRNA is not predictive of MMR (low – 60% vs high 78 p=0.241) CMR (low – 45% vs high 55 p=0.456) EFS (low – 55% vs high 70 p=0.315) or mutation development (low – 18% vs high 14% p=0.666). These data indicate that the level of OCT-1 mRNA is not sufficiently discriminating to predict response and progression. While our previous studies demonstrated that OA could predict the rate of decline in BCR-ABL over the first 12-24 months, this update demonstrates for the first time, that this assay can identify nearly all patients (>80%) who fail to achieve MMR in the long term. Most importantly OA is also strongly predictive of resistance and progression events. Functional assessment of OCT-1 Activity provides prognostic information that is more discriminating than assaying the level of OCT-1 mRNA. This long term study reinforces the notion that OA is an important predictive variable in CP-CML patients treated with IM. It provides further evidence that OA is a critical variable to consider in future trials of imatinib and a key factor to enable individualization of imatinib dose to optimize the long term outcome for CML patients.

	The % of patients achieving:- By 60 months			
	MMR	CMR	EFS	Mutations
Overall (n=56)	71	50	64	12
Low OA 0-7.2 ng/200,000 cells (n=29)	55	34	52	21
High OA >7.2 ng/200,000 cells (n=27)	89	67	78	4
p-value	***0.007***	***0.032***	***0.038***	***0.047***

Table 1: **60 month analysis of the % of patients achieving MMR, CMR and EFS rates.**

	Event Free Survival	Transformation Free Survival	Overall Survival
Q1 (<4.0ng/200,000cells:n=14)	43	79	79
Remainder (n=42)	71	100	98
p-value	***0.023***	***0.002***	***0.009***

Table 2: Overall, Event and Progression Free Survival in Q1 compared to the remainder of the cohort.

5.4. **[508] A Gene Expression Signature of CD34+ Cells to Predict Major Cytogenetic Response in Chronic Phase Chronic Myeloid Leukemia Patients Treated with Imatinib: Potential Involvement of Beta-Catenin in Drug Resistance**. *McWeeney* In chronic phase chronic myeloid leukemia (CML) patients the lack of a major cytogenetic response (MCyR, <36% Ph+ metaphases) to imatinib within 12 months indicates failure and mandates a change of therapy. To identify biomarkers predictive of imatinib failure we performed gene expression array profiling of CD34+ cells from two independent cohorts of imatinib-naïve chronic phase CML patients. The learning set consisted of retrospectively selected patients with a complete cytogenetic response (CCyR) or >65% Ph-positive metaphases within 12 months of imatinib therapy. Based on ANOVA p<0.1 and fold difference >I1.5I we identified 885 probe sets with differential expression between responders and non-responders, from which we extracted a 75-probe set minimal signature (classifier) that separated the two groups. Upon application to a prospectively accrued validation set, the classifier correctly predicted 88% of responders and 83% of non-responders. Bioinformatics analysis and comparison with published studies revealed highly significant overlap of the resistance signature with CML progression signatures. Consistent with this, differential expression of selected resistance genes was confirmed by qPCR on CD34+ cells from an independent set of patients with chronic phase vs. blast crisis. Furthermore, upon analysis of promoter sequences and comparison with a library of physical beta-catenin targets [generated by serial analysis of chromatin occupancy (SACO) in the HCC-116 colon cancer cell line] we find evidence that b-catenin may be a master regulator of resistance genes. Consistent with this, preliminary chromatin immunoprecipitation (ChIP) data on primary cells support physical beta-catenin binding to selected resistance genes, suggesting that Wnt/beta-catenin activation may be involved in primary cytogenetic resistance as well as disease progression. Conclusion: Our data suggest that chronic phase CML patients destined to fail imatinib have more advanced disease than evident by morphological criteria. Our classifier may allow directing more aggressive therapy upfront to the patients most likely to benefit, while sparing good-risk patients from unnecessary toxicity. The potential role of beta-catenin in the regulation of resistance genes suggests that targeting Wnt/beta-catenin signaling may be useful to overcome resistance.

5.5. **[509] Predictive Factors for Response and Outcome in Patients (pts) Treated with Second Generation**

Tyrosine Kinase Inhibitors (2-TKI) for Chronic Myeloid Leukemia in Chronic Phase (CML-CP) Post Imatinib Failure. *Jabbour.* The availability of 2-TKIs has provided new therapeutic options for pts with CML post imatinib failure. We assessed the predictive factors of outcome of pts in CML-CP treated with 2-TKI. A total of 128 pts with CML-CP after imatinib failure treated with dasatinib (n=76) or nilotinib (n=52) were analyzed. Median age was 56 years (range, 21-83). The median duration of CP (CML diagnosis to start of 2-TKI) was 66 months (range, 2-241). Their best response to imatinib was complete hematologic response only in 33%, and cytogenetic response in 55% (23% complete, 16% partial, 15% minor). 4 pts were refractory to imatinib, 3 had unknown response, and 8 were intolerant. At the start of 2-TKI, 94 (73%) pts had active disease. 23% had clonal evolution (CE), and 73% had more than 90% Philadelphia positivity. The median follow-up time was 39 months (range, 15-61) from the start of the 2-TKI. At the time of last follow-up, 108 of the 128 pts (85%) were alive, 86 (67%) in CP on 2-TKI therapy; 17 pts had died. Responses to 2-TKI are shown in Table 1. In the univariate analysis (UA) for event-free survival (EFS), factors associated with poor EFS were splenomegaly, anemia (hemoglobin ≤12g/dL), lack of any cytogenetic response to previous imatinib therapy, ≥ 90% Philadelphia-positive (Ph+) metaphases at the start of 2-TKI therapy, nilotinib therapy, and high sokal risk score disease. In the subsequent multivariate analysis (MVA), splenomegaly, anemia (hemoglobin ≤12g/dL), lack of any cytogenetic response to previous imatinib therapy, and ≥ 90% Ph+ metaphases were selected as independent factors associated with poor EFS. Factors associated with poor overall survival (OS) in the UA were CE, performance status (PS) ≥1, and high sokal risk score at the start of 2-TKI therapy. In the MVA, only CE and a PS ≥1 were selected as independent poor prognostic factors for OS. High hemoglobin level (≥12g/dL), 0% bone marrow blasts, previous cytogenetic response to imatinib therapy, ≤90% Ph+ metaphases, and low sokal risk score were associated with the achievement of a major cytogenetic response (MCyR) by 12 months of therapy with 2-TKI in the UA. In the subsequent MVA for response, the lack of any cytogenetic response to imatinib therapy, anemia (hemoglobin ≤12g/dL) and ≥90% Ph+ metaphases at the start of 2-TKI therapy were selected as poor predictive factors for 12-month MCyR. Pts with 0, 1, 2, or 3 adverse factors had a 12-month probability of achieving a MCyR with 2-TKI therapy of 85%, 79%, 35%, and 14%, respectively. Based on these findings, we developed a score model that included the factors identified as independent predictive for a MCyR by 12 months of therapy with 2-TKI. Three prognostic risk groups are proposed for the new score model: 1) low score (no adverse factors; 16% of pts), in which pts have a 12-month probability of achieving a MCyR of 85%, after therapy with 2-TKI; 2) intermediate score (1-2 adverse factors; 67% of pts), in which pts have a 12-month probability of achieving a MCyR of 56%; and 3) high score (3 adverse factors; 17% of pts), in which pts have a 12-month probability of achieving a MCyR of 14% (Table 2). This score model predicts significantly for EFS (p=0.003) with a trend for OS (p=0.18). In conclusion, the outcome of pts post imatinib failure treated with 2-TKIs is dependent on previous cytogenetic response to imatinib, absence of anemia, and disease burden at the start of therapy. Pts with no previous cytogenetic response to imatinib therapy with anemia and high disease burden have a low likelihood of responding to 2-TKI with poor EFS, and therefore should be offered alternative treatment options.

Table 1. Response to second generation TKIs

	% Response			
	Overall	**Dasatinib**	**Nilotinib**	
Parameter	**N=128**	**N=76**	**N=52**	**p-value**
CHR	83	86	78	0.34
Cytogenetic response	70	70	71	0.86
Major	59	58	60	0.85
Complete	52	53	52	0.94
36-month EFS	58	64	48	0.03
36-month OS	82	85	78	0.27

CHR = complete hematologic response; EFS = event-free survival; OS = overall survival.

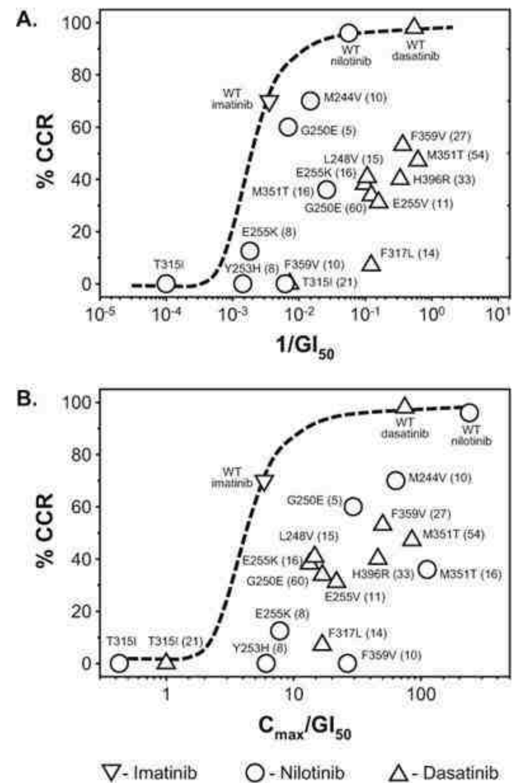
Table 2. Predicted outcome by number of risk factors (No cytogenetic response on imatinib, Hgb<12, %Ph>90 at the 2-TKI)

Risk (# risk factors)	Number patients (%)	MCyR 12m (%)	OS 36m (%)	EFS 36m (%)
Low (0)	20 (16)	85	95	85
Intermediate (1-2)	85 (67)	56	82	57
High (3)	21 (17)	14	72	38

CG=cytogenetic; CCGR=complete cytogenetic response; m=month; OS=overall survival; EFS=event-free survival; NA=not applicable.

5.6. **[510] Comparative in Vitro Cellular Data Alone Is Insufficient to Predict Clinical Responses and Guide Choice of BCR-ABL Inhibitor to Treat Imatinib-Resistant Chronic Myeloid Leukemia.** *Laneuville.* Background: Recently, Redaelli et al (*J Clin Oncol.* 2009;27:469) compared the *in vitro* inhibitory activity of imatinib, dasatinib, nilotinib, and bosutinib against 18 mutant forms of BCR-ABL (expressed in transfected Ba/F3 cells) associated with imatinib resistance and proposed a chart to assist in the selection of second-generation tyrosine kinase inhibitors (2TKIs) for the treatment of imatinib-resistant CML associated with mutations. However, the predictability of this chart has neither been clinically evaluated nor does it take into account other important clinical factors (*e.g.* pharmacokinetics (PK)/pharmacodynamics) that may impact response rates to 2TKIs in the presence of mutations. The purpose was to assess the impact of 2TKIs' *in vivo* plasma levels on the *in vitro* GI_{50} data, and to determine if *in vitro* GI_{50} data with or without plasma levels correlates with

observed clinical responses in imatinib-resistant patients (pts) with mutations. Methods: To enable appropriate comparison of the activity of 2TKIs against specific mutations we modified the original *in vitro* GI_{50} data by adjusting it to include an estimate of *in vivo* C_{max} exposure data for each 2TKI. Further refinement was achieved by calculating the C_{max}/GI_{50} values for each agent and normalizing these against imatinib vs *wild-type* BCR-ABL. To assess the correlation between patient response and *in vitro* GI_{50} data, the previously published CCyR rates for pts with specific mutations were plotted according to *in vitro* GI_{50} values alone and against the adjusted C_{max}/GI_{50} values. Results: The adjusted C_{max}/GI_{50} data suggest that nilotinib delivers the most potent inhibition of most BCR-ABL mutations *in vivo*, with dasatinib being the next most potent. However, when either *in vitro* GI_{50} data alone or the modified C_{max}/GI_{50} data are considered, there is poor correlation of clinical responses to both nilotinib and dasatinib against several of the mutations *in vivo* (Figure). Overall, activity of 2TKIs against all mutations was less than expected based on original *in vitro* GI_{50} or C_{max}/GI_{50} calculations of systemic exposure. For example, the G250E mutation has similar systemic exposure to nilotinib as the F359V mutation as indicated by C_{max}/GI_{50}, but substantial differences are observed in the CCyR rate (60% vs 0%). For dasatinib, the same was observed for the F317L and L248V mutations which have similar exposures to dasatinib but have different CCyR rates (7% vs 41%). Similarly, several mutations with comparable exposure to nilotinib and dasatinib had substantial differences in CCyR rates, suggesting that other factors were influencing responses. For example, the G250E mutation was considered moderately sensitive to both nilotinib and dasatinib based on the adjusted C_{max}/GI_{50}; however, CCyR rates on nilotinib were much higher (60%) compared with dasatinib (34%). Similarly, the E255K mutation was considered moderately sensitive to both agents based on the adjusted C_{max}/GI_{50}; however, CCyR rates on dasatinib were much higher (38%) compared with nilotinib (13%). Conclusions: This analysis illustrates the limitations of *in vitro* inhibition data alone or in combination with PK exposure data in the selection of 2TKI therapy for imatinib-resistant pts with mutations. The current analysis still does not consider parameters such as protein binding and intracellular influx/efflux, among a variety of other clinical factors that could further influence response rates. This tool is also not useful for pts with mutations of unknown *in vitro* sensitivity, which may represent 15% of all pts with mutations. Currently, clinical responses remain the best approach for selection of 2TKIs in pts with mutations, with only a small subset of mutations having low sensitivity mutations existing for each 2TKI. Other factors, such as patient medical history, comorbidities, and the agents' safety profiles, are also important in selection of 2TKIs.



6 *Managing Resistance and Residual Disease [643-648]*

6.1. **[643] A Phase 1 Trial of Oral AP24534 in Patients with Refractory Chronic Myeloid Leukemia and Other Hematologic Malignancies: First Results of Safety and Clinical Activity against T315I and Resistant Mutations.** *Cortes*. AP24534 is an orally available multiple tyrosine kinase inhibitor (TKI) designed using a structure-based approach as a pan-BCR-ABL inhibitor. AP24534 potently inhibits the enzymatic activity of BCR-ABL-T315I, the native enzyme and all other tested variants. It also inhibits survival of cell lines expressing these BCR-ABL variants with IC50s of < 40 nM, and inhibits Flt3 and c-Src. We report here preliminary results from our ongoing phase 1 clinical trial. The objectives of this study are to assess the safety of AP24534, establish a maximum tolerated dose and schedule for further investigation, and provide preliminary assessments of clinical activity. The trial employs an open-label dose escalation design. Patients (pts) with hematologic malignancies refractory to treatment, ECOG status ≤ 2, adequate hepatic and renal function, and normal cardiac function are eligible and receive a single daily oral dose of AP24534. Thirty-two pts (16 males) have been enrolled, median age 63 years (range 31-79). Diagnoses include 27 CML (19 chronic [CP], 4 accelerated [AP], 4 blast phase [BP]), 1 Ph+ ALL, 2 myelofibrosis, 1 myeloma, 1 MDS. BCR-ABL mutation status in 28 Ph+ pts included 5 pts with no mutation, 12 T315I (8 at entry, 4 by history), 3 F317L, (2 at entry, 1 by history), 2 M351T, and 1 each L273M/F359V, G250E, E279K, F359C, L387F and E453K. Prior therapies in CML pts included imatinib (100% of pts), dasatinib (94%), nilotinib (53%), interferon (47%), chemotherapy (41%), and investigational (65%); 83% were resistant to 3 or more TKIs. Pts have been treated at the following dose levels: 2 mg (3 pts), 4 mg (6 pts), 8 mg (7 pts), 15 mg (8 pts), 30 mg (7 pts), and 60 mg (1 pt). 21 pts remain on study. Of 23 pts in the 4 highest (8-60 mg) dosing cohorts, 19 remain on study. Median time on study drug is currently 3.4 months (range 5 days to 10 months). At the time of this report, preliminary safety and efficacy data are available for 31 patients. No DLTs have been observed. The most common drug-related adverse events (AE) were nausea (15%), fatigue and dry eye (12% each), anorexia, arthralgia, dizziness, dry mouth, headache, hot flush, myalgia, vomiting (8% each), and QTc prolongation (1 pt in 2 mg, 1 pt in 4 mg cohort). Grade 3 or 4 thrombocytopenia occurred in 36% pts (18% entered with thrombocytopenia), and grade 3 or 4 neutropenia in 41% (18% entered with neutropenia). Both types of hematologic toxicity were more frequent in pts with advanced stages of CML or baseline cytopenia. Pharmacokinetic data demonstrate that the half life of the drug is 19-45 hours. The relationship of Cmax to dose is linear over the dosing range. The Cmax on day 1 at the 30 mg dose is approximately 55 nM. After one 28-day cycle, the AUC is 2.5- to 3-fold higher than single dose AUC. PD data demonstrate inhibition of CrkL phosphorylation at doses of 8 mg and higher. Overall best

hematologic response was complete hematologic response (CHR) in 16 of 18 CP pts (88%), 5 of whom had CHR on entry, major hematologic response (MHR) in 2 of 4 AP pts, and no response in 4 BP and 1 ALL pts. Cytogenetic responses were 4 complete cytogenetic responses (CCyR) and 2 PCyR. Of the 12 patients with T315I mutations, 9 remain on study without progression. Best hematologic response in the T315I subset was CHR in 5 of 6 CP pts (83%), 2 of whom had CHR on entry, MHR in 2 of 2 AP pts, resolution of extramedullary symptoms in 1 BP pt, and no response in 2 BP and 1 ALL pts. Nine of 12 T315I pts are evaluable for CyR: 2 (1 CP, 1 AP) achieved CCyR after 2 and 5 months (at 4 mg and 15 mg), and 1 CP achieved PCyR after 3 months at 15 mg. Of 3 pts with dasatinib resistant F317L, 1 discontinued for an unrelated AE (CNS ischemia), and 2 remain on study in CP at 8 and 15 mg. One pt with nilotinib resistant F359C remains on study with CHR, CCyR, and major molecular response after 4 months at 15 mg. *Conclusions*: No DLTs have been observed at doses up to 30 mg AP24534. PK and PD demonstrate that blood levels at 30 mg exceed those needed for in vitro inhibition of resistant mutant BCR-ABL isoforms, including T315I. Preliminary analysis reveals evidence of clinical antitumor activity in patients with resistance to approved second-line TKIs dasatinib and nilotinib, including pts with the T315I mutation of BCR-ABL.

NOTES ON PRESENTATION

44 patients. Median 62 years. 18 315 patients including 10 CP. DLT was pancreatic @ 60mg. 64% still on therapy. 16% had disease progression. ¾ tox: 40% thrombo. 43 neutro. Lipase and amylase 5%.

20 cp eval. 45% MCR, 25% CCR.
315s
7CP: 3 got MCR, 2 got CCR. All had CHR.

6.2. **[644] Safety and Efficacy of Subcutaneous-Administered Omacetaxine Mepesuccinate in Imatinib-Resistant Chronic Myeloid Leukemia (CML) Patients Who Harbor the Bcr- Abl T315I Mutation – Results of An Ongoing Multicenter Phase 2/3 Study.** *Cortes*. Background: Omacetaxine is a first-in-class cetaxine with clinical activity against Ph+ CML and a mechanism of action independent of tyrosine kinase inhibition. Currently available tyrosine kinase inhibitors (TKIs) have not demonstrated activity in CML patients (Pts) who harbor the Bcr-Abl T315I mutation. Study Goals: To evaluate the safety and efficacy of subcutaneously (SC) administered omacetaxine in Pts with imatinib (IM)-resistant T315I+ Ph+ CML. Methods: Eligible Pts include adult CML Pts in chronic, accelerated, or blast disease phase (CP, AP, BP) with a confirmed Bcr-Abl T315I mutation and resistance to IM therapy. Induction schedule: 1.25 mg/m^2 SC omacetaxine twice daily for 14 days every 28 days until hematologic response. Maintenance dosing: 1.25 mg/m^2 SC omacetaxine twice daily for 7 days every 28 days. Study Results: To date, 90 Pts have been enrolled, with data available for analysis on 66 Pts (40 CP, 16 AP and 10 BP). The median age was 58 yrs (19-83) with 70% male Pts and a median disease duration of 54 mo (5-285). All Pts failed prior IM therapy, and 79% failed two or more prior TKIs. The presence of baseline T315I mutation was confirmed in all Pts. Baseline clonal evolution was evident in 10 (25%) CP, 6 (38%) AP, and 7 (70%) BP Pts. Eight CP Pts entered the study in CHR. The median follow-up for all Pts was 6.4 mo (0.2 to 29.6). Efficacy: In CP Pts, CHR was achieved in 26 Pts and maintained in 8 Pts for an overall CHR rate of 85%; the median duration of CHR was 7.7+ mo (1.7 to 23.6). Overall cytogenetic response was 27.5% with 6 (15%) Pts achieving a major cytogenetic response (MCyR, 4 complete, 2 partial). The median duration of MCyR was 6+ mo (0.8 to 16.1). Major molecular response was achieved in 15% of Pts and a reduction of baseline T315I mutated clone occurred in 56.7% of CP Pts. In AP Pts, overall hematologic response was achieved in 6 (37.5%) Pts with 5 CHR and 1 return to chronic phase (RCP). Median duration of response was 3.9+ mo (1.7 to 14.8). One AP Pt achieved a complete cytogenetic response; duration 1.9+ mo. In BP Pts, overall hematologic response was achieved in 3 (30%) Pts with 2 CHR and 1 RCP. The median overall survival for CP Pts has not been reached and 35 (88%) Pts were alive at the time of data cut- off. The median overall survival was 18.8 mo for AP and 1.8 mo for BP Pts. Median time to progression was 11.2, 3.1, and 1.2 mo for CP, AP, and BP Pts, respectively. Safety: Grade 3/4 related events occurred in 45 of 66 (68%) Pts. The most commonly reported events were thrombocytopenia (58%), anemia (36%) and neutropenia (33%). Non-hematologic toxicities were primarily grade 1/2 with the most frequently reported events of diarrhea (44%), fatigue (35%), pyrexia (32%), nausea (26%), and asthenia (21%). Grade 3/4 non-hematologic toxicities were uncommon with no events occurring in >5% of Pts and infection (3%) the most common event. Treatment delays occurred in approximately 50% of the Pts with median duration of approximately 12 days for all disease phases and cycles (CP=12, AP=10, and BP=12 days). The primary causes of delay were thrombocytopenia, neutropenia and pancytopenia. Sixteen deaths occurred during the study (5 CP, 4 AP, and 7 BP).Three deaths (1 CP, 1 AP, and 1 BP) were considered to have a possible relationship to omacetaxine: sepsis, pancytopenia, and sudden death with unknown cause, respectively. Conclusions: Omacetaxine administered by subcutaneous injection produces durable hematologic and cytogenetic responses with a safety profile consisting mainly of hematologic toxicities. Omacetaxine may provide a treatment option for this patient population who currently has no available approved drug therapies.

6.3. **[645] Impact of Allogeneic Stem Cell Transplantation as Salvage Therapy After T315I Mutation Detection in Chronic Myeloid Leukemia (CML) and Ph+ Acute Lymphoblastic Leukemia (ALL) Patients.** *Nicolini. Background:* The development of a BCR-ABL T315I mutation is associated with a poor prognosis and limited therapeutic options. The impact of the mutation on the outcome of stem cell transplantation (SCT) is unknown. *Aim:* To describe the overall survival (OS) of CML patients in any phase and Ph+ ALL patients who received an allogeneic SCT after developing a T315I mutation after exposure to tyrosine kinase inhibitors (TKI). *Methods*: We conducted a retrospective, multi-center observational study of 222 CML and *de novo* Ph+ ALL patients who developed a T315I mutation between 1999 and 2008. Data from the medical records of 33 patients (15% of all patients in the registry) from 9 countries (USA, France, Italy, Germany, Denmark, Singapore, and the UK) who received an allogeneic SCT after T315I mutation detection were included in this study. *Results*: At the time of diagnosis, the median age was 39 years (range, 16-67); 70% were male; 26 patients were in CML CP, 1 in

CML AP, 2 in CML BC, and 4 had Ph+ ALL. The median time between diagnosis and TKI treatment start was 3 months (range, 0-125), between diagnosis and T315I mutation detection was 28 months (range, 3-131), and between TKI treatment start and T315I mutation detection was 19 months (range, 2-64). Five (15%) patients had TKIs as frontline therapy. At the time of T315I detection, 10 patients were in CML CP, 7 in CML AP, 12 in CML BC, and 4 had Ph+ ALL. Hydroxyurea (alone or combined with other treatments) was the most common 1st line treatment (55%) after T315I mutation detection. The median time from T315I mutation detection to SCT was 3 months (range, 0.3-28). At the time of transplant, the median age was 42 years (range, 22-68); 8 patients were in CML CP, 7 in CML AP, 14 in CML BC and 4 had Ph+ ALL; 32 patients received 1 SCT and 1 received 2 SCTs after T315I mutation detection. The source of stem cells was peripheral blood (53%), bone marrow (35%), cord blood (6%), and unknown (6%). 82% were matched donor and 18% were unmatched. The median follow-up time from SCT was 7 months and 15 (55%) patients had died by their last follow-up. The OS of CML CP and CML AP patients was much better than CML BP and Ph+ ALL patients (Fig. 1; logrank, p=.050). The 1-yr OS rates (95% CI) from SCT were 69% (21-91%) for CML CP, 71% (26-92%) for CML AP, 16% (3-39%) for CML BC, and 33% (1-77%) for Ph+ ALL; and the 3-yr OS rates (range) was 69% (21-91%) for CML CP, 71% (26-92%) for CML AP, 0 for CML BC, and 0 for Ph+ ALL. *Conclusion*: These results suggest that the survival of patients harboring a T315I mutation and treated with allogeneic SCT is dependent on the disease phase at the time of SCT. SCT is the treatment of choice for these CML patients, particularly those in CP and AP. Fig. 1. Overall survival from Allogeneic SCT

6.4. **[646] Inhibition of Bcl-2/Bcl-X_L Promotes Apoptosis in Blast Crisis CML Including Quiescent Primitive Progenitor Cells Regardless of Cellular Responses to Tyrosine Kinase Inhibitors.** *Mak.* The advent of imatinib, a Bcr-Abl tyrosine kinase inhibitor revolutionized the treatment for patients with CML. Development of resistance, limited activity in blast crisis CML, and more importantly, insensitivity of quiescent primitive CD34$^+$ CML progenitor cells are evolving problems facing this therapy. Antiapoptotic Bcl-2 proteins were known to be highly expressed in Bcr-Abl expressing cells and inhibition of Bcl-2/Bcl-X_L by the selective inhibitor ABT-737 was reported to augment the killing of tyrosine kinase inhibitors in CML cells. However, its effect on quiescent primitive CD34$^+$ CML progenitor cells is unknown. To investigate the effect of activating the apoptotic machinery in quiescent primitive CD34$^+$ CML progenitor cells, which are resistant to current therapies, we first compared the expression of antiapoptotic proteins in proliferating and quiescent primitive CD34$^+$ CML progenitor cells. Cells obtained from patients with blast crisis CML were stained with the fluorescent 5-(and 6-) carboxy-fluorescein diacetate succinimidyl ester, a cell proliferation tracking dye, and cultured *in vitro* for 4-6 days. Cells were then stained with CD34 antibody and FACS sorted into proliferating and quiescent CD34$^+$/PI- CML progenitor cells. RNA levels of antiapoptotic proteins in these two cell populations (n=8) were determined by real-time RT-PCR: quiescent and proliferating primitive CD34$^+$ CML progenitor cells expressed similar levels of Bcl-2, Bcl-X_L, Mcl-1, and XIAP implying that like total blast cells, quiescent primitive CD34$^+$ CML progenitor cells may also be sensitive to agents targeting these proteins. We next treated 5 samples obtained from patients with blast crisis CML with ABT-737 and measured apoptosis in total CD34$^+$ cells, proliferating CD34$^+$ cells, and quiescent CD34$^+$ cells. All 5 patients were resistant to or relapsed from imatinib and nilotinib and/or dasatinib treatments and they were insensitive to imatinib *in vitro* as expected. However, cells from 4 patients were sensitive to ABT-737, in bulk blasts and in both proliferating and quiescent CD34$^+$ CML cell compartments: % specific apoptosis with 100 nM of ABT-737=40.8±7.7, 38.4±8.5, 40.0±5.1, respectively at 24 hours. Interestingly, when ABT-737 was combined with imatinib, cell death was greatly enhanced in cells from all 5 patients in all cell compartments (combination index=0.059±0.032, 0.041±0.025, 0.111±0.042, respectively). Furthermore, we showed previously, that triptolide, an antitumor agent from a Chinese herb, induces apoptosis in both proliferating and quiescent primitive CD34$^+$ CML progenitor cells by decreasing Mcl-1 which is a resistant factor for ABT-737, XIAP, and Bcr-Abl protein levels (Mak D. et al., MCT in press). When ABT-737 was combined with triptolide, a significant increase of cell death was found in total CD34$^+$ and proliferating as well as quiescent primitive CD34$^+$ CML cells with combination index at EC50=0.57, 0.55, and 0.56, respectively in cells from the 5 patients suggesting a high degree of synergism. In summary, Bcl-2, Bcl-X_L, Mcl-1, and XIAP are equally expressed in proliferating and quiescent primitive CML cells and targeting Bcl-2/Bcl-X_L promotes death of blast crisis CML cells, tyrosine kinase inhibitor resistant CML cells, and quiescent primitive CD34$^+$ CML progenitor cells. Researches suggest that the combination of apoptosis inducing agents and tyrosine kinase inhibitor is a novel strategy to overcome tyrosine kinase resistance, eradicate quiescent primitive CML progenitor cells, and improve current therapy for patients with CML.

6.5. **[647] Interferon Alpha 2a (IFN) Maintenance Therapy After Imatinib Plus IFN Induction Therapy in Chronic Myeloid Leukemia (CML) Induces Stable Long-Term Molecular Remissions and Is Associated with Increased Proteinase 3 (PR3) Expression and the Presence of PR1-Specific T-Cells.** *Burchert.* Imatinib is a selective and very potent inhibitor of the BCR/ABL kinase. It induces ongoing complete cytogenetic remissions in the vast majority of chronic phase CML patients. However, BCR/ABL persistence is the rule despite ongoing imatinib therapy. This suggests that imatinib will not to cure CML and raises concerns about emerging imatinib resistance, long-term imatinib tolerability and compliance to therapy. We previously suggested that a combination of imatinib and immunotherapy by IFN may additionally control CML via induction of autologous cytotoxic T-cell (CTL) responses, such as those directed against the leukemia-associated antigen proteinase 3 (PR3). For example, induction of PR1-CTL which recognize PR3 on CML blasts was previously shown to be associated with IFN-, but not imatinib response. Indeed, we could recently demonstrate on a cohort of 20 newly diagnosed CML patients that low dose of IFN maintenance therapy alone was able to maintain or improve remissions obtained by a prior imatinib/IFN combination treatment (A. Hochhaus et al. , ASH 2007). After a median time of IFN maintenance therapy of 1.2 years 80% of the patients remained or improved molecular remission. Here we report a significantly longer follow up of these patients and translational studies to examine markers of IFN response. Twenty pts (14 m, 6 f; median age 45, range 23-74 yrs) with low (n=13), intermediate (n=6), and high risk (n=1) according to the Hasford score risk calculation have been investigated. Imatinib therapy had been administered for 2.4 yrs (0.2-4.9), combined with PEG-IFNa2a (Pegasys®, n=17) or IFN a2a (Roferon®, n=3). Maintenance therapy consisted of PEG-IFN (n=16) or IFN (n=4). Dose was adjusted according to response and tolerability and ranged between 135 µg PEG-IFN every 3 weeks to 180

µg PEG-IFN every week, or alternatively 2 to 5 * 3 Mill IU IFN/week. Imatinib was terminated due to side effects (n=5) or upon personal request of the patients after informed consent (n=15). At the time of imatinib withdrawal, two pts were in complete molecular remission (CMR) and 15 pts in major molecular remission (MMR). After a median observation time of 2.8 yrs (range 0.5-4.5), 15 pts were in MMR, 5 of them in CMR. Thus, the number of MMR patients increased from 2 at baseline to 5 after two years. Five patients relapsed within 0.4 years (range, 0.2-0.8) after imatinib discontinuation, but were rescued with imatinib, re-establishing molecular remission. Side effects to maintenance IFN were minor. We also studied putative markers of IFN response. IFN therapy was associated with an increase in the expression of PR3, and in the presence of auto-reactive PR1-CTL. PR1-CTL frequencies were prospectively assessed without prior in vitro amplification. In one of five assessable patients PR1-CTL were detected prior to imatinib withdrawal, but in four of seven assessable patients during IFN maintenance therapy. Longitudinal measurements of PR-1 CTL counts suggested an inhibition of the expansion of PR1-CTL by imatinib, implying that an optimal CTL expansion may occur preferentially in the absence of imatinib. This would explain the conversion to a CMR status in some patients only after imatinib withdrawal. Together, IFN maintenance after a prior imatinib/IFN induction therapy may be an effective alternative to permanent imatinib therapy, because it enables to safely discontinue imatinib even in those patients that have not achieved a CMR at the time of pausing imatinib. Induction of a PR1-specific CTL response by IFN may contribute to the particular efficacy of IFN after CML-debulking by imatinib.

6.6. **[648] BCR-ABL Derived Peptide Vaccine in Chronic Myeloid Leukemia Patients with Molecular Minimal Residual Disease During Imatinib: Interim Analysis of a Phase 2 Multicenter GIMEMA CML Working Party Trial.** *Bocchia.* Introduction: Imatinib (IM) 400mg daily is the standard treatment for chronic myeloid leukemia (CML) patients and a complete cytogenetic response (CCyR) is achieved in the majority of patients within one year of treatment. In addition, a considerable number of patients reach a major molecular response (i.e BCR-ABL/ABL ratio <0.1%) but BCR-ABL transcript is still measurable in most of treated patients revealing the persistence of a minimal residual disease (MRD). In a previous small pilot study, vaccinations with p210 b3a2-derived fusion peptides in IM treated CML patients appeared to induce both a peptide specific immune response and a reduction of residual disease surviving IM. Methods: To investigate the efficacy of this immune based targeted approach in a larger cohort of patients we designed a phase 2 multicenter study (GIMEMA CML0206) employing 5 p210 b3a2-derived peptides (CMLVAX100 vaccine) in CML patients with at least 18 months of IM treatment and persistence of molecular residual disease. Each vaccination consisted of CMLVAX100 plus 2 doses of GM-CSF as immunological adjuvant. Treatment schedule included 6 biweekly vaccinations (immunization phase) followed by 3 monthly boosts (reinforcement phase) and 2 tri-monthly boosts (maintenance phase). The primary endpoint of the trial was to assess the rate of response (patients showing a reduction by at least 50% of peripheral blood BCR-ABL/ABL ratio compared to the individual prevaccine level) evaluated after immunization and reinforcement boosts (evaluation after 6 months,) and persisting at the 9th month (after 10th vaccination). Secondary endpoints included the rate of undetectable transcript at any time after immunization and the rate of peptide-specific immune response induced by the vaccinations. Patients population: At present 57/69 planned patients have been enrolled and 43/57 are evaluable for response. Twentyseven are males and 16 are females with a median age of 56.5ys (range 29-78). At diagnosis, 25/43 (58%) patients presented with low, 15/43 (35%) with intermediate and 3/43(7%) with a high Sokal risk. Twenty-one out of 43 patients (49%) started standard IM treatment while in late chronic phase (CP) after a median time from diagnosis of 29 months during which they mainly received alpha interferon therapy. On the contrary 22/43(51%) patients started IM immediately after diagnosis. All patients entered the vaccination protocol after at least 18 months of IM treatment and the median time of exposure to this tyrosin kynase inhibitor before peptide vaccination was 54 months (range 23-100). All patients had obtained a CCyR before entering the study (after a median time of 6 months of IM treatment) and were still in CCyR at enrollment with a median duration of CCyR of 47 months. All patients started vaccination with persisting measurable molecular disease in peripheral blood (any level of BCR/ABL transcript). Results: Current interim analysis shows that vaccinations (a total of over 400 shots) were very well tolerated, with CMLVAX100-GMCSF toxicity consisting exclusively of some redness and itching at the site of injection and with only 4/43 patients (9%) experiencing a mild fever. Regarding immune response induced by vaccination, 29/43 patients (67%) showed a significant in vitro b3a2-peptide-specific CD4+ T cell proliferation. With respect to MRD response, we observed a reduction of at least 50% of pre-vaccine BCR-ABL/ABL values after 6 months of treatment (i.e. after 9 vaccinations) in 22/43 (51%) patients and the reduction was confirmed in 14/29 (48%) patients who reached the 9th month evaluation (i.e. after 10 vaccinations); 14/43(32%) patients had at least one documented undetectable transcript during this time period. In 2/43 patients we observed a significant raise of BCR-ABL transcript level, after 3 and 18 months from starting vaccinations with subsequent loss of CCyR. Conclusions: CMLVAX100 vaccine appears to induce a reduction of long lasting molecular MRD surviving IM in about half of vaccinated CML patients, thus confirming preliminary results. If this BCR-ABL-specific immune control of MRD will have a substantial impact on the rate of BCR-ABL mutations, disease evolution and ultimately survival needs longer observation time to be determined.

7 Late-breaking abstract – nilotinib vs imatinib [LBA-1]

7.1. **[LBA-1] Nilotinib Demonstrates Superior Efficacy Compared with Imatinib in Patients with Newly Diagnosed Chronic Myeloid Leukemia in Chronic Phase: Results From the International Randomized Phase III ENESTnd Trial.** ***Saglio***. Background: Nilotinib is a highly potent and the most selective inhibitor of BCR-ABL, the only proven molecular target for CML therapy. ENESTnd (Evaluating Nilotinib Efficacy and Safety in Clinical Trials-Newly Diagnosed Patients) is a phase 3, randomized, open-label, multicenter study comparing the efficacy and safety of 300 or 400 mg bid nilotinib with 400 mg qd imatinib in patients (pts) with newly diagnosed Ph+ CML in chronic phase (CML-CP). Methods: 846 pts with newly diagnosed Ph+ CML-CP, diagnosed within 6 mos, and stratified by Sokal risk score, were randomized 1:1:1 to nilotinib 300 mg bid (n=282), nilotinib 400 mg bid (n=281), and imatinib 400 mg qd (n=283) arms. The primary endpoint was rate of major molecular response (MMR) at 12 months (mos). All pts had a minimum of 12 mos of treatment or discontinued early; median follow-up was 14 mos. MMR was defined as a value of ≤ 0.1% of BCR-ABL/ABL ratio on the International Scale. Molecular

response was assessed by RQ-PCR at baseline, monthly for 3 mos and every 3 mos thereafter. Samples were analyzed at a central PCR laboratory. The major secondary endpoint was rate of complete cytogenetic response (CCyR) by 12 mos based on bone marrow cytogenetics. Results: Baseline demographics, disease characteristics, and Sokal scores were well balanced among the 3 arms; pts with high-risk Sokal scores were 28% in all arms. Median dose intensities of nilotinib delivered were 592 mg/day for 300 mg bid and 779 mg/day for 400 mg bid; imatinib dose intensity was 400 mg/day. Overall, 84%, 82%, and 79% of pts remained on the study for 300 mg bid nilotinib, 400 mg bid nilotinib, and 400 mg qd imatinib, respectively. Rates of MMR at 12 mos (Table) were superior for nilotinib 300 mg bid compared with imatinib 400 mg qd (44% vs. 22%, P < .0001) and also for nilotinib 400 mg bid compared with imatinib 400 mg qd (43% vs. 22%, P < .0001). Median time to MMR among pts who achieved MMR was faster for nilotinib 300 mg bid (5.7 mos) and nilotinib 400 mg bid (5.8 mos) compared with imatinib 400 mg qd (8.3 mos). Rates of CCyR by 12 mos were significantly higher for both nilotinib at either 300 mg bid compared with imatinib 400 mg qd (80% vs. 65%, P < .0001) and for nilotinib 400 mg bid compared with imatinib 400 mg qd (78% vs. 65%, P = .0005). Overall, progression to advanced disease was lower for nilotinib 300 mg bid (2 pts) and nilotinib 400 mg bid (1 pt) compared with imatinib 400 mg qd (11 pts). Overall, both drugs were well-tolerated. Rates of discontinuation due to adverse events or laboratory abnormalities were 7% for nilotinib 300 mg bid, 11% for nilotinib 400 mg bid, and 9% for imatinib 400 mg qd. Pts were monitored for QT prolongation and LVEF. No patients in any treatment arm showed a QTcF interval > 500 msec. There was no decrease from baseline in mean LVEF anytime during treatment in any arm. The study is ongoing. Conclusions: Nilotinib at both 300 mg bid and 400 mg bid induced significantly higher and faster rates of MMR and CCyR compared with imatinib 400 mg qd, the current standard of care in pts with newly diagnosed CML. Nilotinib was effective across all Sokal scores. After only one year of treatment, both nilotinib arms resulted in a meaningful clinical benefit compared to imatinib, with reduction of transformation to AP/BC. Nilotinib exhibited a favorable safety and tolerability profile. The superior efficacy and favorable tolerability profile of nilotinib compared with imatinib suggests that nilotinib may become the standard of care in newly diagnosed CML.

Molecular and Cytogenetic Response Rates Overall and by Sokal Score (ITT)

	Treatment Arm		
	Nilotinib, 300 mg BID (N = 282)	Nilotinib, 400 mg BID (N = 281)	Imatinib, 400 mg QD (N = 283)
MMR, n (%)			
At 3 mos	25 (9%)	14 (5%)	2 (1%)
At 6 mos	93 (33%)	83 (30%)	34 (12%)
At 12 mos	125 (44%)* P < .0001	120 (43%)* P < .0001	63 (22%)
Overall MMR†, n (%)	161 (57%)	153 (54%)	86 (30%)
High-risk Sokal , MMR (12 mos), n/N (%)	32/78 (41%)	25/78 (32%)	13/78 (17%)
CCyR, n (%)			
By 6 mos	188 (67%)	177 (63%)	126 (45%)
By 12 mos	226 (80%)* P < .0001	219 (78%)* P = .0005	184 (65%)
Estimated rate of progression to AP/BC (12 mos), (%)	<1% P = .0095‡	<1% P = .0037‡	4%

* Cochran-Mantel-Haenszel test stratified by Sokal risk group vs imatinib for 12 months.
† MMR at any time point up to data cut-off.
‡ Stratified (by Sokal risk group) log-rank test vs imatinib for time to AP/BC.

8 New Insights into the Molecular Biology of CML [853-858]

8.1. **[853] DNA Ligase III Alpha and (Poly-ADP) Ribose Polymerase (PARP1) Are Therapeutic Targets in Imatinib-Resistant (IR) Chronic Myeloid Leukemia (CML).** *Tobin*. Therapy with the tyrosine kinase inhibitor imatinib, targeting the constitutively active BCR-ABL kinase has been remarkably successful in Philadelphia chromosome-positive (Ph+) CML, but resistance to tyrosine kinase inhibitors is a growing clinical problem, prompting the search for new therapeutic targets. BCR-ABL expression leads to increased reactive oxygen species (ROS), repair errors and genomic instability. We have previously shown that an error-prone alternative non-homologous end-joining (ALT NHEJ) pathway involving PARP1 and DNA ligase IIIa/XRCC1 is upregulated in Ph+ CML, providing a mechanism for the repair errors and genomic instability. To determine whether ALT NHEJ components may be novel therapeutic targets in IR CML, we characterized two IR cell lines (P210Mo7eIR, Baf3P210IR) for DSB repair abnormalities. Both IR cell lines demonstrate significantly higher levels of DSBs and NHEJ abnormalities (P<0.05) compared with their imatinib-sensitive (IS) counterparts. Notably, whereas steady state levels of the ALT NHEJ components DNA ligase IIIa and PARP1 are increased in IS P210Mo7e and Baf3P210 cells, compared with parental Mo7e and Baf3, the levels of these proteins are increased even further in the IR cells. Presence of increased DNA ligase IIIa and PARP1 levels in the IR cell lines suggests that these enzymes may be targets for therapy using the DNA ligase inhibitors that we have previously identified and PARP1 inhibitors, which have been used successfully in the treatment of cancers with DSB repair defects. Initial tests for cytotoxicity in BCR-ABL-positive cell lines and parental controls showed that the DNA ligase inhibitor L67, which specifically inhibits DNA ligase I and III , is cytotoxic in BCR-ABL-positive cells and parental controls at concentrations of >10 µM, and that cytotoxicity is not influenced by BCR-ABL1

expression. Therefore, we examined the effect of a subtoxic concentration of L67 (0.3 μM) in the presence or absence of the PARP1 inhibitor Nu1025 (Calbiochem) at 50 μM in IR versus IS and parental cells. Combined treatment with L67 and Nu1025 significantly ($p<0.001$) reduces survival of IR cells compared with IS and parental controls, which were not significantly affected. To determine whether cells from CML patients that are resistant to imatinib are also sensitive to the combination of DNA ligase and PARP inhibitors, we next tested primary bone marrow mononuclear cells (BM MNC) from 6 CML patients with IR disease, compared with normal BM MNC. Cells from 3 of the 6 patients demonstrated a significant decrease in colony survival in response to the combination of DNA repair inhibitors, similar to the sensitivity demonstrated by the two IR cell lines studied. Interestingly, the patient demonstrating the highest sensitivity to the combination of DNA repair inhibitors had significantly increased levels of both DNA ligase IIIa and PARP1, whereas patients demonstrating less sensitivity had increased levels of either DNA ligase IIIa or PARP1, compared with normal BM MNC. Importantly, sensitivity to the DNA repair inhibitors is not correlated with mutations in BCR-ABL because the BCR-ABL mutation T315I that is found in Baf3P210IR cells when overexpressed in Baf3 cells has no effect on colony survival following drug treatment. Together, our results suggest that the process of acquiring IR may select for cells with high levels of PARP1 and DNA ligase IIIa and/or may upregulate ALT NHEJ pathways. Thus, patients with high levels of these proteins are likely to benefit from therapy using inhibitors of ALT NHEJ.

8.2. **[854] Suppression of Bcr-Abl Expression in CML by A Panel of miRNAs.** *Iraci*. It has been proposed that most protein-encoding genes may be regulated by small multifunctional RNAs which can control transcript turnover and/or protein translation (siRNA and miRNA). Specifically, miRNAs have been shown to act predominantly at the level of translation by blocking the access or sliding of ribosomes to mRNAs (3'UTR). Several studies have shown that multiple miRNAs can be disregulated in tumours as compared to normal tissues. For example, the miR-17-92 cluster is up regulated in CML, suggesting their involvement in leukemiagenesis (Venturini et al., Blood 2007). However, a role of miRNA in preventing tumor development and progression has also been suggested. Bueno et al have shown that hsa-miR-203 can specifically target BCR-ABL and reduce its expression in CML derived cell lines (Bueno et al., Cancer Cell 2008). Since multiple miRNAs seem to act in a combinatorial fashion to regulate mRNA translation, we hypothesised that other miRNAs in addition to hsa-miR-203 might be involved in BCR-ABL expression control. To test this hypothesis we conducted a search for miRNAs specifically targeting Bcr-Abl, using the miRBASE program to scan human genome (http://microrna.sanger.ac.uk/). This search identified 15 miRNAs potentially able to target the BCR-ABL 3' UTR. Further investigation showed that only hsa-miR-451, hsa-miR-515-3p and hsa-miR-760 had a sufficiently high score to predict genuine interactions with the 3'UTR of BCR-ABL and therefore only these miRNAs were utilized for further analyses. Initially, the three miRNAs were transfected in K562 Ph+ cells, together with hsa-miR-203 as a positive control and a scrambled miRNA used as a negative control. BCR-ABL expression was monitored at both mRNA (qRT-PCR) and protein level (Western Blotting). Our results demonstrated that BCR-ABL mRNA expression was unaffected by miRNAs, whereas protein expression was significantly reduced. Considering the combinatorial function of miRNAs on their target mRNAs, we also tested whether a pool of the four identified miRNAs could be effective in suppressing BCR-ABL expression. In those experiments, the molar concentration of each miRNA was a quarter of that used for a single miRNA transfection. The results have shown that the pool of miRNAs worked efficiently in suppressing Bcr-Abl expression, which suggested a cooperative function of the four miRNAs in controlling both the expression and translation of Bcr-Abl. To further confirm that miRNAs directly targeted BCR-ABL, the 3'UTR of the gene was cloned downstream of a reporter renilla luciferase gene. The reporter was co-transfected in HEK293 cells together with single miRNAs or a pool of them, and luciferase activity was quantified. Those results show that the presence of each miRNAs significantly reduced the luciferase activity as compared to that obtained by transfecting cells with a scrambled miRNA. These experiments therefore confirmed a direct effect of the four miRNAs on the 3'UTR of BCR-ABL. Again the "pool" of miRNAs showed the strongest effect on the luc reporter expression. Through an additional deletion analysis we mapped the regions of 3'UTR of BCR-ABL targeted by the four miRNAs, in order to confirm that the predicted binding regions of miRNAs are critical to mediate repression of BCR-ABL. In conclusion, our study identified three new human miRNAs having a potential to specifically target BCR-ABL and suppress its translation and expression in CML cells. BCR-ABL, which plays a critical role in CML, is effectively suppressed by TK inhibitors, exemplified by Gleevec and our findings provide a rationale to exploit miRNA as an alternative therapeutic approache which could further improve CML treatment, or to complement TK inhibitors in an effort to eradicate minimal residual disease. Supported by: Novartis Oncology, Clinical Development, TOPS Clinical Correlative Studies Network

8.3. **[855] Suppression of RISC-Independent Decoy and RISC-Mediated mRNA Base-Pairing Activities of MicroRNA-328 Is Required for Differentiation-Arrest and Enhanced Survival of Blast Crisis CML Progenitors. *Eiring***. MicroRNAs (miRs) and heterogeneous ribonucleoproteins (hnRNPs) are post-transcriptional gene regulators that bind mRNA in a sequence-specific manner. We have reported that a) hnRNP-E2 suppresses CEBPA mRNA translation and inhibits myeloid maturation of bone marrow (BM) progenitors from chronic myelogenous leukemia patients in myeloid blast crisis (CML-BCCD34+; Perrotti et al, Nat Genet 2002); and b) miR-328 expression is lost in myeloid CML-BCCD34+ progenitors (n=6) and its restored expression at physiological levels rescues granulocytic differentiation and impairs clonogenic potential of primary BCR/ABL+ blasts (Eiring et al, ASH 2007). Here we show by Northern blot, real-time PCR, and microarray analyses that miR-328 levels increase during granulocytic differentiation of normal human CD34+ and mouse Lin- BM progenitors, but not during differentiation towards erythroid, megakaryocytic or monocytic lineages. BCR/ABL uses the same MAPKERK1/2-hnRNP-E2 signaling pathway to suppress both C/EBP and miR-328, as pharmacologic or shRNA-mediated inhibition of these molecules restored miR-328 expression in BCR/ABL+ cells. In fact, two functional C/EBP binding sites are present in the miR-328 promoter region and C/EBP interacts in vivo with these regulatory elements to enhance miR-328 transcription. Importantly, we also show that restored maturation of BCR/ABL+ blasts requires direct interaction of hnRNP-E2 with the C-rich regions of miR-328. Indeed, RNA-immunoprecipitation (RIP) assays demonstrated that miR-328 directly binds to hnRNP-E2 independent of the RNA-induced silencing complex (RISC). Furthermore, ectopic miR-328, but not miR-181b, resulted in decreased in vivo binding of hnRNP-E2 to the uORF/spacer

region of CEBPA mRNA, thereby releasing CEBPA from hnRNP-E2 translation inhibition and rescuing C/EBPa-driven neutrophil maturation (decoy activity). Differentiation of miR-328-expressing CML-BCCD34+ blasts (88.8±2.4% post-mitotic cells) correlated with induction of C/EBPa protein expression, whereas CEBPA mRNA and hnRNP E2 protein levels remained unchanged. The existence of a direct miR-328/hnRNP-E2/CEBPA interplay was formally demonstrated in vitro using RRL-directed translation assays and in vivo using the 6.15 clone of 32D-BCR/ABL cells that do not express endogenous CEBPA mRNA and require ectopic C/EBP (wt-uORF-CEBPA) for differentiation. Addition of miR-328, but not miR-330, to hnRNP-E2-containing RRL reactions increased newly synthesized 35S-C/EBPa levels by >100%. Likewise, forced miR-328 expression in vivo resulted in decreased hnRNP-E2 binding to CEBPA mRNA, induction of C/EBPa protein but not mRNA and rescued granulocytic differentiation of 6.15-wt-uORF-CEBPA but not vector-transduced 6.15 cells. While hnRNP-E2 was not found in complex with basic RISC components (Dicer, TRBP2 and Ago2), RIP assays detected miR-328 associated to Dicer and Ago2 in miR-328-expressing cells, suggesting that it also acts through canonical RISC-dependent base-pairing with mRNA targets. Indeed, we identified the BCR/ABL-regulated PIM1 serine-threonine kinase as a bona fide miR-328 target in BCR/ABL+ cells. Ectopic miR-328 suppressed PIM1 protein but not mRNA levels, and this effect required integrity of the miR-328 binding site present in the PIM1 3'UTR. Forced expression of a wild-type but not kinase-deficient PIM1 lacking the 3'UTR into miR-328-expressing cells fully rescued BCR/ABL clonogenicity, suggesting that miR-328-induced PIM1 suppression accounts for reduced survival of miR-328-infected BCR/ABL+ blasts. To show that miR-328 acts on PIM1 in a RISC-dependent manner, we mutated the miR-328 seed sequence (miR-328-Mut) while retaining its C-rich character. Similar to wild-type miR-328, miR-328-Mut efficiently interacted with hnRNP-E2, restored C/EBPa protein expression and rescued granulocytic differentiation, but was unable to silence PIM1 in 32D-BCR/ABL cells, indicating that the C-rich character of miR-328 is essential for its decoy activity, while its seed sequence integrity is necessary for RISC-dependent pairing to mRNA targets. Thus, the discovery of dual activities for miR-328 not only adds a new layer of complexity to the mechanisms regulating CML disease progression, but also highlights the ability of miRNAs to alter mRNA metabolism by acting as molecular decoys for RNA-binding proteins.

8.4. **[856] Mono/Oligoclonal T and NK Cells Are Common in Philadelphia Chromosome Positive (Ph+) Leukemia Patients at Diagnosis and Expand During Successful Tyrosine Kinase Inhibitor Therapy.** *Kreutzmann*. Introduction. Central to current treatment of Ph+ leukemia patients are tyrosine kinase inhibitors (TKIs), which predominantly target the BCR-ABL1 kinase in malignant cells. However, broader-spectrum 2nd generation TKIs, such as bosutinib, dasatinib and nilotinib, also inhibit off-target kinases with important physiological functions. Several in vitro studies have implied that TKIs may have immunosuppressive effects by suppressing activation and proliferation of effector lymphocytes. In contrast, we recently observed immunostimulation during dasatinib therapy in the form of marked expansion of clonal cytotoxic lymphocytes (T- and NK cells) resulting in chronic LGL-type lymphocytosis in peripheral blood (PB). The prevalence, detailed molecular background and clinical implications of clonal lymphocytes during TKI therapy are currently unknown. The aim of this study was to comprehensively analyze clonality and evolution of lymphocyte clones during TKI therapy. Patients and methods. The study population included patients with Ph+ leukemia, both CML (n=28) and ALL patients (n=4) on dasatinib (n=23) and imatinib (n=9) therapies. In addition, samples from 12 healthy controls and diagnostic samples from the nine imatinib treated patients were analyzed. Lymphocyte clonality was determined by analysis of PB mononuclear cells (MNC) for clonal T cell receptor (TCR) and d gene rearrangements by 18 primer pairs covering most known clonal TCR and d rearrangements. Upon positive reaction in heteroduplex analysis, the purified PCR products were sequenced. If clonal rearrangement was observed, allele-spesific PCR primers were designed to allow for quantitative follow-up of lymphocyte clones in each patient. Results. Sequencing-confirmed clonal TCR rearrangement was observed only in 1 of 12 healthy controls and no TCR d gene rearrangements were found in this group. Surprisingly, 7 of 9 (78%) CML patients showed clonal TCR rearrangements at diagnosis. In 3 patients the clonal rearrangement was detected in the TCR d genes, in 7 patients in the TCR genes and 3 patients had rearrangemens both in TCR d and genes. After one year of imatinib treatment the same clones could be detected in 5 of the 7 patients (71%). Although clonal cells were observed, none of the imatinib patients had signs of a concomitant lymphoproliferative disorder and the distribution of lymphocyte subclasses was normal. Next, 23 patients treated with dasatinib were studied, 10 without (LGLneg) and 13 with PB LGL lymphocytosis (LGLpos) including T- or NK-cell expansions. In all LGLpos dasatinib patients (including patients with a CD3neg NK-cell expansion) clonal TCR or d rearrangements were found. In LGLneg dasatinib patients the prevalence of TCR rearrangements was 80%. LGLpos patients had more often clonal rearrangements in TCR d genes (62%) than LGLneg patients (10%). No differences in clonal rearranged TCR genes (77% vs. 80%) were detected. Most patients displayed more than one clonal TCR rearrangement. Quantitative follow-up of LGLpos patients revealed that the expansion of a single predominant lymphocyte clone accounted for LGL lymphocytosis. Intriguingly, quantitative follow-up of lymphocyte clones by PCR showed that the observed clones existed at low levels already before start of dasatinib therapy during imatinib treatment, but no lymphocyte expansions were then seen. Sorting of lymphocytes showed that clonal cells resided in the CD8+ and CD4+ T-cell populations and, strikingly, also among CD16/56+CD3neg NK cells. All dasatinib patients with NK cell expansions (n=3) showed TCR d rearrangements in their NK cells. Conclusions. Clonal lymphocytes could rarely be found in healthy controls. In contrast, they were frequently present in CML patients at diagnosis and persisted during TKI therapy. In a distinct subgroup of dasatinib treated patients, clonal cells massively expanded during successful therapy. Clonal TCR rearrangements were detected in CD4+, CD8+ and, unexpectedly, also in NK cells. The epitopes and function of clonal, CML-associated lymphocytes are under investigation. Previous studies showed that clonal expansions during dasatinib were associated with excellent, long-lasting therapy responses in advanced leukemia. We therefore hypothesize, that the clonal lymphocytes present at CML diagnosis may be anergic anti-leukemic cells and part of the immune escape mechanisms inherent to leukemogenesis and that dasatinib therapy can reverse this anergy.

8.5. **[857] Treatment with Tyrosine Kinase Inhibitors May Impair the Potential Curative Effect of Allogeneic Stem Cell Transplantation.** *Jedema*. Tyrosine kinase inhibitors (TKI) like imatinib and dasatinib are the current treatment of choice for patients with chronic myeloid leukemia (CML). Although most patients enter a complete remission during

treatment, cure of the disease is usually not achieved since recurrence of the disease is seen in the majority of patients upon discontinuation of the treatment, indicating that the leukemic stem cell is not efficiently targeted. Furthermore, in accelerated phase and blast crisis of CML TKI treatment only results in temporary control of the disease. In these situations allogeneic stem cell transplantation (allo-SCT) and application of donor T cells may be the only curative treatment. Besides the direct anti-leukemic effect of allo-SCT, alloreactive T cells recognizing CML (progenitor) cells, and the formation of immunological memory may lead to effective lifelong immune surveillance. Therefore, we investigated whether the leukemic cells persisting during TKI treatment are susceptible targets for the anti-leukemic effect mediated by donor T cells after allo-SCT and whether continuous TKI treatment may have an additive effect during the immunological intervention. To investigate the anti-leukemic effect of the two strategies, CD34+ positive CML cells were isolated from bone marrow, and labeled with the fluorescent dyes CFSE or PKH to allow monitoring of single cell proliferation. CML cells were exposed to imatinib (1-100µM) or dasatinib (0.01-50nM), and/or to CD8+ alloreactive cytotoxic T lymphocyte (CTL) clones in the presence of proliferation-inducing cytokines. The number, phenotype, and proliferative status of the CML cells persisting after single and combined interventions were measured by quantitative flowcytometric analysis. In the absence of therapeutic interventions the majority of CD34+ CML cells entered proliferation. However, a small population of CD34+ CML stem cells residing in the non-dividing peak could be identified despite the addition of cytokines. Addition of imatinib or dasatinib resulted in efficient dose-dependent induction of cell death of the leukemic cells (99% lysis by 25µM imatinib or 10nM dasatinib). However, the population of quiescent CD34+ CML stem cells was not affected. Moreover, the number of cells present in the non-dividing population increased 2-fold compared to the non-treated controls at the highest TKI concentrations, indicating additional growth arrest of a population of proliferating CML precursor cells. We next tested the capacity of different HLA-A2-restricted CD8+ CTL clones to kill non-treated or imatinib or dasatinib treated CML cells. Whereas the proliferating CD34+ CML precursors were efficiently lysed, the population of quiescent stem cells was capable of withstanding CTL exposure. Detailed phenotypic analysis revealed significant downregulation of HLA-A2 and the adhesion molecules CD49d and CD58 on these quiescent cells, probably resulting in the impaired ability of these target cells to form a high avidity interaction with the T cells. The increased population of non-dividing cells as a result of the TKI pretreatment showed similar resistance to T cell induced cell death, indicating that TKI treatment may even diminish the anti-leukemic effect of allo-SCT. In the absence of therapeutic control, as mimicked by the removal of T cells and TKI from the cultures, outgrowth of the leukemic cells re-occurred, illustrating their capacity to give rise to a relapse of the disease. Next, we analyzed the effect of TKI treatment on T cell survival and functionality. Whereas resting primary T cells were insensitive to TKI treatment, T cells activated by either polyclonal stimulation with anti-CD3/CD28 beads or stimulation with allogeneic stimulator cells died after TKI exposure at similar concentrations as the leukemic cells. In conclusion, TKI treatment results in selection of a population of quiescent leukemic stem cells showing cross-resistance to CTL-induced cell death, most likely due to their inability to form a high avidity interaction. Moreover, T cells actively participating in the anti-leukemic immune response after allo-SCT are suppressed by TKI. These data indicate that continuous TKI treatment may potentially hamper the curative effect of allo-SCT.

8.6. **[858] Characterization of Leukemia-Initiating Cells in a Transgenic Model of Chronic Phase Chronic Myelogenous Leukemia (CML).** *Zhang*. In normal hematopoiesis, only a small population of lin-Sca-1+c-kit+ (LSK) cells with Flt3-CD150+48 immunophenotype has long-term hematopoietic stem cell (LT-HSC) capacity, whereas Flt3-CD150+CD48+ and Flt3-CD150-CD48+ LSK cells represent more differentiated multipotent progenitors (MPP1 and MPP2) without long-term engrafting capacity. Despite extensive investigation into BCR-ABL induced leukemogenesis, the impact of BCR-ABL expression on LSK subpopulations and the specific subpopulation with leukemia-initiating capacity remain unknown. Targeted expression of the BCR-ABL gene in murine hematopoietic stem and progenitor cells (HSPC), using a Tet-regulated SCL promoter, results in development of a chronic phase CML-like disorder (Blood 105:.324, 2005). Mice consistently develop leukocytosis, splenomegaly and expansion of bone marrow (BM) myeloid progenitors and primitive LSK cells following induction of BCR-ABL expression. Here we employed the SCL-tTA-BCR/ABL mouse model to investigate the effect of BCR-ABL expression on HSPC populations. BCR/ABL expression resulted in a 3-fold increase in granulocyte-macrophage progenitors (GMP) and a 1.5-fold increase in LSK cell numbers compared with non-induced controls, whereas numbers of common myeloid progenitors (CMP) and megakaryocyte-erythrocyte progenitors (MEP) were reduced. Despite expansion of total LSK cells, the number of LT-HSC was markedly reduced in the BM of BCR-ABL expressing mice (610±246 vs. 4,038±982). In contrast, an increase in MPP1 (6,150±1,813 vs. 3,185±1,247) and MPP2 (39,580±14,079 vs. 25,115±7,090) was seen. BCR-ABL mRNA expression was confirmed in each population by RT-PCR, with highest levels of expression seen in MPP cells. In vivo EdU labeling demonstrated increased cycling of LSK cells from BCR/ABL expressing mice compared to controls. We observed a vast increase in the number of GMP (11-fold), CMP (10-fold), MPP (4.5 fold) and LT-HSC (2.5 fold) in the spleen of BCR/ABL mice compared to controls. Since the functional potential of HSPC cannot be determined solely on the basis of cell surface markers, we also studied the ability of transplanted populations to generate leukemia in recipient mice. SCL-tTA/BCR-ABL transgenic mice were crossed with GFP transgenic mice to facilitate tracking of transplanted cells. Only LSK cells, but not CMP or GMP, from BM and spleen of BCR-ABL expressing mice were capable of generating CML-like disease and long term engraftment (>16 weeks) in recipient mice. The leukemic phenotype of donors was recapitulated in recipient mice; and leukemia could be transplanted to secondary and tertiary recipients. Further analysis revealed that the subpopulation of cells with a LT-HSC phenotype (Flt3-CD150+CD48-) within the LSK population was capable of generating CML-like disease and long term engraftment in recipient mice. Consistent with the diminished LT-HSC numbers demonstrated by flow cytometry, the frequency of functional HSC within the LSK population, as measured by competitive repopulation limiting dilution assays, was reduced in BCR-ABL expressing mice (1 in 234) compared to control mice (1 in 14). Interestingly, not all transplanted mice with long-term engrafted BCR-ABL-expressing cells developed leukemia. We determined that 1 in 6 Flt3-CD150+CD48- LSK cells possessed repopulation activity, whereas only 1 in 80 cells was capable of initiating leukemia in transplanted mice within 20 weeks, indicating that only a subset of BCR-ABL+ cells with long-term repopulating potential has leukemia-initiating capacity. In summary, BCR-ABL expression is associated with significant reduction in LT-HSC and expansion of MPP and GMP in the BM, and a marked increase in LT-HSC, MPP,

CMP and GMP in the spleen of transgenic mice. Reduced LT-HSC numbers in BM may be explained by increased proliferation of BCR-ABL-expressing HSC and their enhanced egress from the BM to extramedullary locations such as the spleen. BCR-ABL expressing LT-HSC demonstrated long term engraftment and secondary transplantation capacity. However, only a fraction of BCR-ABL-expressing long-term repopulating cells has leukemia-initiating capacity, suggesting that additional cell intrinsic or extrinsic factors besides BCR-ABL expression may play a role in determining their leukemogenic potential.

9 New Trends in Management [859-864]

9.1. **[859] Discontinuation of Imatinib Therapy After Achieving a Molecular Response in Chronic Myeloid Leukemia Patients.** *Mahon*. Background. Imatinib (IM) has greatly improved survival rates in chronic myeloid leukemia* (*CML). However, all patients (pts) must continue treatment for an unknown period of time. A pilot study of the first pts who discontinued IM therapy was previously reported (Rousselot et al. Blood 2007;109:58–60). The multicentre study « Stop Imatinib » (STIM) was initiated in July 2007 in order to evaluate the persistence of complete molecular remission (CMR) after stopping IM, and to determine the factors that could be associated with CMR persistence. Methods. Inclusion criteria were IM treatment duration of at least 3 years and sustained CMR. Sustained CMR was defined as BCR-ABL/ABL levels below a detection threshold corresponding to a 5-log reduction (undetectable signal using RQ-PCR) for at least 2 years. Molecular relapse, defined as RQ-PCR positivity, was taken into account if confirmed in two successive assessments. In cases of molecular relapse, pts were re-challenged with IM at 400 mg daily. Results. From the pilot study, 8 among 15 patients are still in CMR with a median follow up of 42 months (range 37-49). The number of patients enrolled in the STIM study was 69. 34 patients had received interferon alpha (IFNa) prior to IM and 35 pts were de novo. Median follow-up (range) was 17 months (6-24). 37 pts relapsed (loss of CMR) within the first 6 months and two patient relapsed after more than 6 months (M7 ,M18). At M12, the probability of remaining in CMR was 45% (95% CI: 33-56%). For previously treated with IFN (n=34) this probability was 44% (95% CI: 27-59%) versus 46% (95% CI: 29-61%) for de novo pts (p=0.93, overall). All patients in molecular relapse were sensitive again after imatinib re-challenge (decreasing BCR-ABL level, achievement CMR again). Male pts had a better probability of survival without molecular relapse (p=0.02) and a trend was observed for the low Sokal risk group (p = 0.06). Peripheral NK cells counts prior to IM discontinuation were significantly lower in relapse pts (mainly cytotoxic cells CD56dim) as compared to the non relapse pts (p=0.005). Conclusions. We have confirmed that CMR can be sustained after discontinuation of imatinib with a long follow-up, particularly in male patients and in pts with cytotoxic NK cells in their peripheral blood. Using stringent criteria, it is possible to stop treatment in patients with sustained CMR, even in those treated with IM as a single agent.

9.2. **[860] Phase II Multicentric Explorative Study of Intermittent Imatinib (IM) Treatment (INTERIM) in Elderly Patients with Ph+ Chronic Myeloid Leukemia (CML) Who Achieved a Stable Complete Cytogenetic Response (CCgR) with Standard IM Therapy.** *Russo*. Background: Elderly CML patients treated with Imatinib (IM) in early chronic phase (CP) have similar cytogenetic response and survival compared with younger patients, but they show a lower compliance to standard IM therapy (400 mg/day). Aims: The aim of the study is to investigate if CCgR that has been achieved with standard (daily administration) IM therapy can be maintained with the same dose of IM given intermittently (INTERIM). Methods: The study population is represented by elderly patients (³ 65 years old) with Ph+ CML and with stable CCgR after at least 2 years of standard IM therapy (daily administration). IM is given at the same dose that was given at the time of enrollment by the following intermittent schedule: 1 week on / 1 week off for the 1st month; 2 weeks on / 2 weeks off for the 2nd and 3rd month; 1 month on / 1 month off from the 4th month thereafter. In cases of loss of CCgR INTERIM was stopped and standard therapy (daily administration) was resumed. After 12 months, the patients who are in continuous CCgR are advised to continue the intermittent study schedule and to be followed indefinitely. The CgR status was evaluated at baseline (by conventional cytogenetics on bone marrow and FISH on peripheral-blood) and every 3 months during the study (only by FISH on peripheral-blood). If FISH (% of Ph+ cells) increased more than 1% in two consecutive examinations, evaluation of marrow cells metaphases was performed to confirm the loss of CCgR and to check for additional cytogenetic abnormalities. Quantitative molecular assessment of BCR-ABL transcript by RQ-PCR on peripheral blood was due at baseline and every 3 months during the study and mutational analysis of ABL was performed in case of loss of CCgR. Results: One-hundred and fourteen patients have been considered eligible, but 17 (15%) refused to enter into the protocol. Out of 97 enrolled patients, 87 started INTERIM, 5 patients (5%) went off the study for major protocol violation before the 3rd month and, at present, 82 patients are ongoing. Of these 82 patients, 52, 30 and 11 completed the 3rd, 6th and 9th month, respectively. The preliminary results of the first 6 months are here reported. The distribution of patients according to FISH results is shown in Fig. 1. Only 1/68 pts (at 6th month) showed an increased >1% in Ph+ cells by FISH but he maintained a CCgR when checked by conventional cytogenetic. As showed in Fig. 2, 96 to 87% of patients maintained a major molecular response MMR (≤0,1) according to International Scale (IS). Conclusions: This study is trying to test the minimum effective dose of Imatinib to maintain the CCgR in elderly CML patients with stable CCgR. The preliminary results at 6 months do not show negative trends both for cytogenetic and molecular response. Therefore, the study is ongoing and all patients are expected to complete the trial time (12 months).

9.3. **[861] Safety and Efficacy of Subcutaneous-Administered Omacetaxine Mepesuccinate in Chronic Myeloid Leukemia (CML) Patients Who Are Resistant or Intolerant to Two or More Tyrosine Kinase Inhibitors – Results of A Multicenter Phase 2/3 Study.** *Cortes*. Background: Omacetaxine is a first-in-class cetaxine with clinical activity against Ph+ CML and a mechanism of action independent of tyrosine kinase inhibition. The development of TKI resistance and intolerance is an emerging problem and patients (Pts) who have failed multiple TKIs may benefit from an alternative therapy for CML. Study Goals: To evaluate the safety and efficacy of SC omacetaxine in CML Pts who are resistant and/or intolerant to two or more TKIs. Methods: Eligible Pts included adult CML Pts in chronic, accelerated, or blast disease phase (CP, AP,

BP) with resistance and/or intolerance to at least two TKIs. Bcr-Abl mutational analysis was performed at one of 2 central reference laboratories and Pts harboring the T315I Bcr-Abl mutation were enrolled in a separate clinical trial. Induction schedule: 1.25 mg/m2 SC omacetaxine twice daily for 14 days every 28 days until hematologic response. Maintenance dosing: 1.25 mg/m2 SC omacetaxine twice daily for 7 days every 28 days. Study Results: To date, 99 Pts have enrolled, with data available for analysis on 65 Pts (30 CP, 20 AP and 15 BP). The median age was 57 yrs (23-78) with 52% male and a median disease duration of 77 mo (1-197). Nearly all (64/65, 99%) Pts failed prior IM therapy and 57% failed 3 or more prior TKIs. Baseline mutations were identified in 21 (32%) Pts with 10 non-P Loop, 7 P Loop and 4 compound mutations. Baseline clonal evolution was evident in 5 (17%) CP, 8 (40%) AP, and 13 (87%) BP Pts. Six CP Pts entered the study in CHR. The median follow-up for all Pts is 4.0 mo (0.3 to 14.7). Efficacy: In CP Pts, CHR was achieved in 18 Pts and maintained for more than 8 weeks in the 6 Pts enrolled with baseline CHR, for an overall CHR rate of 80%; median duration 4.7+ mo (1.4 to 13). Major cytogenetic response (MCyR) was achieved in 6 (20%) CP Pts (1 complete, 5 partial); median duration 1.6+ mo (0.0 to 2.9). Major molecular response was achieved in 10% of CP Pts. In AP Pts, overall hematologic response was achieved in 15 (75%) Pts; 12 CHR and 3 return to chronic phase (RCP); median duration 2.5+ mo (1.8 to 10.5). One (5%) AP Pt achieved a complete CyR, identified immediately prior to data cut-off and ongoing. In BP Pts, overall hematologic response was achieved in 8 (53.3%); 6 CHR and 2 RCP. Three Pts (2 CP, 1 AP) received bone marrow/stem cell transplants after achieving major cytogenetic response, a therapeutic option not available to them at study enrollment. No deaths occurred in CP Pts. The median overall survival for AP Pts has not been reached and 13 Pts were alive at the time of data cut-off. Median overall survival was 14.5 mo for BP Pts. Median time to progression was 11.1, 5.7, and 2.6 mo for CP, AP, and BP Pts, respectively. Safety: Grade 3/4 related events occurred in 47/65 (72%) of Pts. The most commonly reported events (>15%) were thrombocytopenia (43%), neutropenia (29%), and anemia (22%). Non-hematologic toxicities were generally grade 1/2 with the most frequently reported; diarrhea (32%), nausea (26%), pyrexia (23%), headache (20%), fatigue (19%), vomiting (17%), and asthenia (17%). Grade 3/4 non-hematologic toxicities were uncommon with no events occurring in >5% of Pts and fatigue (3%) the most common event. Treatment delays occurred in approximately 50% of the Pts with median duration of approximately 9 days for all disease phases and cycles (CP=7, AP=11, and BP=12 days). The primary causes of delay were thrombocytopenia, neutropenia and pancytopenia. Deaths occurred in 6 (9.2%) Pts, including 2 (10%) AP pts and 4 (26.7%) BP Pts. Of the deaths, one occurrence in an AP Pt was considered to be possibly related to omacetaxine treatment (febrile neutropenia). Conclusions: Omacetaxine administered by SC injection produced hematologic and cytogenetic responses with a safety profile primarily consisting of hematologic toxicities. This study demonstrated that omacetaxine may be a potential treatment option for CML Pts who have failed multiple TKIs.

9.4. **[862] Optimaization of Imatinib Therapy by Combination. 5 Year Survival and Response Results of the Pilot Phase of the Randomized German CML STUDY IV.** *Pletsch*. Rapid relapse after discontinuation of imatinib, the need for indefinite therapy and residual disease in most patients are the major challenges in management of CML. Combinations of imatinib with IFN simultaneously, or consecutively preceding imatinib, or with araC may improve treatment outcome. The German CML Study Group therefore designed a randomized trial to compare standard imatinib vs. imatinib + interferon alpha (IFN) vs. imatinib + low dose araC vs. imatinib after IFN failure (for low- and intermediate-risk patients, high risk patients received imatinib 800 mg instead). The current evaluation represents the prefinal results of the pilot phase of the trial. Inclusion criteria were newly diagnosed BCR/ABL positive CML in chronic phase (CP). Primary aims are: prolongation of survival (overall, OS, and progression free, PFS), determination of rates of hematologic, cytogenetic and molecular remissions, adverse events (AE) and role of allografting. By the end of 2005, 670 patients were randomized, 13 had to be excluded (no CML (n=3), pregnancy, no CP (n=1 each), imatinib 800 mg (n=8)). Analysis was according to intention to treat. 657 patients were evaluable (174 with imatinib 400 mg, 196 with imatinib+IFN, 158 with imatinib+araC and 129 with imatinib after IFN-failure). 656 patients were evaluable for hematologic, 611 for cytogenetic, and 618 for molecular responses. Patient characteristics of treatment arms were similar for age (median 53 years), sex (40% female), median values for Hb (12.6 g/dl), WBC (66.2/µl), platelets (383/µl) and for Euro risk score (low 35%, intermediate 54%, high 10%). The median dose of imatinib was 400mg/die in all arms, of araC 10 mg per treatment day and of IFN 4.2 Mio I.U./die in the imatinib after IFN arm and 1.8 Mio I.U./die in the imatinib+IFN arm. Median observation time was 57.3 months. 55 patients died, 73 patients were transplanted in 1st CP, 81 patients progressed, 59 patients were switched to second generation TKIs. After 3 years 126 patients (72%) of the imatinib 400mg arm still received the initial therapy as well as 60 patients (30%) of the imatinib+IFN arm and 53 patients (34%) of the imatinib+araC arm. 9 patients (7%) of the imatinib after IFN arm are still on IFN. 5-year OS of all patients is 91%. 5-year PFS of all patients (no death, patient still in first chronic phase) is 87%. 5-year-OS and PFS according to treatment arm are shown in the Table. At 5 years, the cumulative incidences of achieving complete cytogenetic remission or major molecular remission (MMR) as determined by competing risks (death, progression) are not different (Table). Type and severity of adverse events (AE) over a 5-years period did not differ from those reported previously (Table). Hematologic AEs grade III/IV were similar in all therapy arms except leukopenia grade III/IV, which was more frequently observed in the imatinib after IFN arm (14%). Non hematologic AEs were mainly fluid retention, neurological and gastrointestinal symptoms and fatigue. Neurologic symptoms and fatigue were more often reported for the therapy arms with IFN. This analysis shows excellent survival and durable response rates in all arms. Currently, survival in all treatment arms is equal to, or better than in IRIS. To verify possible differences in survival, e.g. imatinib 400 mg vs. imatinib + IFN, longer observation is planned. Although cytogenetic and molecular responses in the imatinib after IFN failure arm at 5 years are inferior to that in the other treatment arms, the question of whether the consecutive therapy with IFN first and imatinib after IFN-failure provides a survival advantage requires long term follow-up. Imatinib in combination with, or after IFN, or with low dose araC are feasible and safe treatment modalities. We expect that the study will optimize and improve therapy outcome in CML.

	Imatinib 400mg	Imatinib+IFN	Imatinib+AraC	Imatinib after IFN
	5-Year Survival and Response Rates			

OS	87%	93%	92%	92%
PFS	84%	91%	88%	84%
CCR	92%	92 %	89%	83%
MMR	83%	78%	80%	70%
	Adverse Events, WHO Grade III/IV			
Anemia	7%	1%	3%	3%
Leukopenia	4%	5%	2%	14%
Thrombocytopenia	5%	6%	6%	6%
	WHO Grade I-IV			
Edema	15%	13%	5%	0%
Neurological	5%	15%	5%	22%
Gastrointestinal	17%	27%	21%	15%
Fatigue	8%	13%	9%	23%

9.5. **[863] Imatinib Is Efficient but Has a Negative Impact On Growth in Children with Previously Untreated chronic Myelogenous Leukaemia (CML) in Early Chronic Phase (CP): Results of the French National Phase IV Trial.** *Millot*. Aims: In order to investigate the efficacy and the safety of imatinib in children and adolescents with untreated Philadelphia-positive CML, the CML working party of the Société Française des Cancers de l'enfant (SFCE) conducted an open label, multicentric phase IV study trial (Clinical Trials.gov.NCT00845221). Patients and methods. Patients less than 18 yrs of age with newly diagnosed CP CML were eligible. Imatinib was administered orally at a dose of 260 mg/m2 which is equivalent in terms of drug exposure to the total dose of 400 mg in adults. Forty four children (64% boys) with a median age of 11.5 yrs (range 10 months-17 yrs) have been enrolled from 15 French pediatric centres between July 2004 and December 2008. Side effects were reported prospectively using the NIH CTCv2.0 criteria. Results. Median follow up is currently 17 months (range:1-67). The complete hematologic response rate was 86% and 98% (ITT) at 3 months and 6 months, respectively. The rate of complete cytogenetic response (CCyR) was 62% (ITT) at 12 months. The rate of major molecular response (MMR) defined as a BCR-ABL/ABL ratio ≤ 0.1% according to the International Scale was 34% (ITT) at 12 months. The median daily dose of imatinib was close to the intended doses, 250 mg/m2 (range 176-405) with a median treatment duration of 16 months (2 w to 67 mo). Three pts (7%) had a dose reduction of more than 25% of the theoretic dose. Nine pts (20%) interrupted imatinib temporarily at least once for a median duration of dose interruption of 12 days (range 2-70). Ten pts (23%) discontinued imatinib. The reasons for discontinuation were the following: allogeneic HSCT in CCyR or MMR (2 pts) according to the investigator choice, adverse event (2 pts: muscle pain 1 pt, liver enzyme elevation 1 pt), disease progression or failure (6 pts: loss of CHR [L248V mutation] 1pt, loss of CCyR [L384M mutation] 1pt, no cytogenetic response 1 pt, no molecular response 1 pt, loss of MMR 2 pts). One of them (loss of hematologic response) transformed to blastic phase shortly after coming off study and died. Grade III or IV toxicity was recorded in 14 pts (32%). GradeIII-IV hematologic toxicity was recorded in 8 pts (18%) including neutropenia in 18% and thrombocytopenia in 5%. Nine pts (21%) developed grade III or IV non-hematologic toxicity: muscle pain (2.5%), arthralgia (5%), weight gain (13%), liver enzyme (GOT/GPT) increase (6.5%). Change of body height was observed during the first year of treatment with imatinib in the 22 pts with a sufficient follow-up: a significant decrease of height standard deviation scores (SDS) was observed with a median of the difference of -0.37 (range, -1.09 to +0.14)($p<0.0001$) between the start of the treatment and 12 months later. Conclusion. Imatinib is efficient in children and adolescent with previously untreated CML in early chronic phase. However, we report for the first time, a negative impact of imatinib on the growth in a cohort of children and adolescents treated with imatinib.

9.6. **[864] Danusertib Hydrochloride (PHA-739358), a Multi-Kinase Aurora Inhibitor, Elicits Clinical Benefit in Advanced Chronic Myeloid Leukemia and Philadelphia Chromosome Positive Acute Lymphoblastic Leukemia.** *Cortes*. Background: Despite the availability of novel ABL tyrosine kinase inhibitors (TKI) in addition to imatinib mesylate, the acquisition of the T315I BCR-Abl mutation remains a major cause of resistance to registered therapeutic compounds. Some patients (Pts) may also fail therapy with ≥ 2 TKI and need additional treatment. PHA-739358 is a small ATP competitive molecule that specifically inhibits Aurora A, B and C kinases. PHA-739358 possesses high affinity binding capacity to both wild-type Abl and Abl/T315I in in vitro assays. Methods: An international, multicenter, open-label, single agent, non-comparative, phase I study is being conducted in adult Pts with advanced Chronic Myeloid Leukemia (Accelerated AP-CML/Blastic phase BP-CML) and Philadelphia-Chromosome Positive Acute Lymphoblastic Leukemia (Ph+ ALL) resistant or intolerant to imatinib and/or 2nd generation c-Abl therapy. Primary objective of the study is to determine the Maximum Tolerated Dose (MTD) and the Dose Limiting Toxicity (DLTs) during the first cycle. Two schedules were planned. Schedule A (PHA-739358 given as daily 3-hrs iv infusion for 7 consecutive days, every 2 wks) is open. Schedule B, not yet started, foresees a more aggressive approach and is currently being amended. Results: Twenty-three Pts with CML and Ph+ ALL have been treated so far (4 with AP-CML; 8 with BP-CML and 11 with Ph+ALL). Five dose levels have been tested: 90 (N=7); 120 (N=4); 150 (N=6); 180 (N=3) and 200 (N=3) mg/m2. Only one DLT occurred at 90 mg/m2 (NCI-CTC AE Gr3 fainting). Presently the MTD has not been defined. Fifteen out of 23 Pts have confirmation of BCR-Abl T315I mutation. A response occurred in 6/14 Pts, including 3 cytogenetic (1 complete, 1 partial, 1 minimal), 5 hematologic, and 1 clinical improvement (reduction in extramedullary disease mass). One severely pretreated (chemotherapy+Imatinib, SCT, Dasatinib and Donor Lymphocyte Infusion) pt with T315I mutated Ph+ALL reached Complete Hematological Response since Cycle (Cy) 4, Complete Cytogenetic Response since Cy 8 and Molecular Response (BCR/ABL undetectable transcripts) since Cy 9. Treatment continues after 10+ months. Last cycles were given every 4 wks due to mild/moderate transaminitis. This Pt at baseline showed: Peripheral Blood Blasts=0%; Bone Marrow (BM) blasts= 33%; % of Ph+ Metaphases = 80%. Two additional Pts (AP-CML and Ph+ ALL) achieved cytogenetic responses, one minimal and one partial reached at Cy2 and

Cys 1-6, respectively. Another Pt (BP-CML) who received multiple transplants (3 times) and progressed after Imatinib, Dasatinib, Bosutinib, Nilotinib and Homoharringtonine, with 4 cycles of PHA-739358 had significant improvement of a large extramedullary lesion in the left neck impeding breathing and swallowing. Significant but transient reduction in the White Blood Cells (8/13 pts) and peripheral blood blast counts (3/13 Pts) were obtained with PHA-739358. An acceptable tolerability and safety profile characterized the study therapy: max non-hematological tox (regardless of causality) was as follows (most frequent Adverse events ≥ 30 %): diarrhoea 57% (CTC Gr 3 one Pt), pyrexia 50%; headache 43% (CTC Gr 3 one Pt); dyspnoea 36% (CTC Gr 3 one Pt) and nausea 36%. Data analysis ongoing. Conclusions: The preliminary results obtained with our study show that PHA-739358 can elicit significant response with clinical benefit in severely pretreated populations of Pts affected with advanced leukemias resistant/intolerant to Imatinib and other 2nd generation TKI.

10 Posters: Biology and Pathophysiology I

If you click on the abstract, you will be taken to the full details on the ASH abstract web site.

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2161. Splice Variant JAK2 Transcript Deleting Exon 14 in Patients with Chronic Myeloproliferative Neoplasms
2162. Conditional Activation of Bcr-Abl Kinase Delineates Time-Dependent Transforming Events in Hematopoietic Cells
2163. Angiogenic Factors in Chronic Myelogenous Leukemia: Evaluation at Diagnosis and After 3 and 6 Months of Treatment with Imatinib
2164. Ph-Negative Hematopoiesis Emerging After Successful Treatment of Chronic Myeloid Leukemia Displays Severe and Persistent Telomeric Loss Which Is Particularly Prominent in Patients with Acquired Cytogenetic Abnormalities
2165. BCR-ABL Tyrosine Kinase Activity Modulates the Phosphorylation, Localization and Function of Interferon Regulatory Factor 5 (irf-5) in Chronic Myeloid Leukemia (CML) Cells
2166. Hypoxia-Adapted CML Cells Are More Primitive Population and Are Eradicated by Glyoxalase-1 Inhibitors
2167. BCL6 Is Required for Leukemia-Initiation and Self-Renewal Signaling in Chronic Myeloid Leukemia
2168. Wnt-Pathway Directed Compound Targets Blast Crisis and Chronic Phase CML Leukemia Stem Progenitors
2169. Imatinib Mesylate and Nilotinib Affect the MHC-Class I Presentation by Modulating the Proteasomal Processing of Antigenic Peptides
2170. Enhanced Jak2 Activation Correlates with Chain Expression Leading to Phosphorylation of Tyrosine 177 of Bcr-Abl
2171. The Oncogenic Kinase Bcr-Abl Directly Regulates Splicing of BclX through a Quaternary Complex Coordinated by Nck-Beta and Sam-68 Adapter Proteins
2172. Targeting IL3 Receptor in Chronic Myeloid Leukemia
2173. Cbl and TET2 Mutations Are Present in Refractory Ph+ Disorders Including Accelerated and Blast Crisis CML and ALL
2174. CD34+ obtained from High Sokal Risk Chronic Myeloid Leukemia (CML) Patients (PTS) Expresses Gene Profiles (GEP) Significantly Different From CD34+ Obtained From Low Sokal Risk Patients
2175. Tyrosine Kinase Proteins profiling of Nilotinib Resistant Chronic Myelogenous Leukemia Cells Unravels a Tyrosine Kinase-Mediated Bypass
2176. High-Resolution Molecular Allelokaryotyping of Chronic Myeloid Leukemia Patients in Blast Crisis by 6.0 SNP-Arrays Shows a High-Frequency of Uniparental Disomy and Focal Copy Number Alterations Affecting the Whole Sequence or Specific Exons of Oncogenes and Tumor Suppressor Genes
2177. Arsenic Trioxide (ATO) Interacts Synergistically with MEK Inhibitors to Induce Apoptosis in STI571-Resistant Bcr-Abl Mutants
2178. IFN- (interferon-gamma) Genotype Predict Cytogenetic and Molecular Response to Imatinib Therapy in Chronic Myeloid Leukemia
2179. A Common but Overlooked Mechanism of BCR-ABL1 Kinase Inhibitor Resistance in Chronic Myeloid Leukemia
2180. Cytokine-Mediated Signaling Is Suppressed in Myeloid Cells and Enhanced in Lymphatic Cells in Patients with Chronic Myeloid Leukemia (CML) – Partial Normalization with Tyrosine Kinase Inhibitors
2181. Physiologic Hypoxia Protects Chronic Myelogenous Leukemia Progenitors From Elimination by Imatinib Mesylate
2182. Depletion of PHLPP1 and 2 by Bcr-Abl Promotes CML Cell Proliferation through the Continuous Phosphorylation of Akt
2183. An Autophagy Inhibitor, Chloroquine, Overcomes An Imatinib-Resistant T315I Mutant of Bcr/Abl, Whose Latent May Exist Before Imatinib Treatment
2184. Inhibition of Wnt/ -Catenin Signaling by AV65 Treatment Caused Cell Cycle Arrest and Induced Caspase-Independent or -Dependent Apoptosis in CML Cells
2185. Peptide-Vaccine Treatment Associated with TKI Therapy in Patients with CML Is Able to Induce Immunologic, Cytogenetic and Molecular Responses: a Single Center Experience with Long-Term Follow up
2186, Genome-Wide Screening for Dominant Modifiers in Drosophila Identified New Cluster of Genes Involved in BCR-ABL Signalling and CML Progression
2187. GATA-2 L359V Mutation Is Exclusively Associated with CML Progression but Not Other Hematological Malignancies and GATA-2 P250A Is a Novel Single Nucleotide Polymorphism
2188. Small Interfering RNA against Bcr-Abl Transcripts Sensitized Mutated T315I Cells to Nilotinib
2189. OCT-1 Activity in CML CD34+ Cells Is Not Predictive of Molecular Response to Imatinib Treatment in CP-CML Patients, Despite the Strong Predictive Value of MNC OCT-1 Activity
2190. NADPH Oxidases Are Important Regulators of Growth and Migration in Myeloid Neoplasms
2191. Chronic Myelogenous Leukemia Cells Contribute to a Bone Marrow-Stroma in In Vivo NOD/SCID Mouse System

11 Posters: Biology and Pathophysiology II

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3245. A New Flowcytometry-Based Method to Discriminate Malignant From Normal Stem Cells in CML
3246. ABT-737 Cooperates in a Strong Synergism with Tyrosine Kinase Inhibitors to Induce Apoptosis of Chronic Myeloid Leukemia Cells
3247. Activity of Serono-AS703569, a Dual Inhibitor of Bcr-Abl and Aurora Kinases in Bcr-Abl Transformed Cells, Is Dependent On Aurora B Inhibition, and Is Not Affected by the Presence of the Highly Imatinib Resistant Bcr-Abl Mutation T315I
3248. BCR-ABL1 Oncogene Down-regulates the Expression of OCT1 in CML
3249. BCR/ABL Requires Fanconi Anemia D2 (FANCD2) Protein to Transform Hematopoetic Stem Cells
3250. Blocking of Cytokine Survival Signals along with Intense Bcr-Abl Kinase Inhibition May Eradicate CML Progenitor Cells
3251. Bmi-1 Overexpression Synergizes with p210-BCR-ABL to Induce Stem Cell and Progenitor Transformation
3252. C-Myc Mediated Regulation of Multidrug Resistance Genes in Chronic Myeloid Leukaemia Cd34+ Cell Progenitors
3253. Deletion of Rac2 Inhibits Proliferation of Chronic Myelogenous Leukemia (CML) Stem Cells and Progenitors (HSC/P) In Vivo and

Promotes Survival of Scl/p210-BCR-ABL Mice
3254. Deletions of the Normal ABL1 at the Non-Translocated Chromosome 9 in CML Associated with TKI Resistance
3255. Downregulation of THAP11 by Bcr-Abl Promotes c-Myc-Mediated CML Cell Proliferation
3256. Downregulation of the Common Cytokine Receptor Subunit Beta c by Omacetaxine in CML: A Potential Molecular Mechanism to Overcome Cytokine-Mediated Resistance against BCR-ABL-Inhibitors
3257. E3 Ligase c-CBL Mediates Ubiquitination-Proteasomal Degradation of BCR-ABL and Therapeutic Effects against BCR-ABL Leukemia.
3258. Frequent Inactivating Mutations of TET2 and CBL Are Associated with Acquired Uniparental Disomy in Atypical Chronic Myeloid Leukemia and Related Disorders
3259. FTY720 but Not Its Immunosuppressive Phosphorylated Form FTY720-P Exerts Anti-Leukemic Activity towards Ph(+) and Ph(-) Myeloproliferative Disorders through Reactivation of the PP2A Tumor Suppressor
3260. Gene Expression Profile in Responsive and Non-Responsive Chronic Myeloid Leukemia Patients Treated with Dasatinib
3261. Genomic Segmental Duplications at the Basis of t(9;22) Rearrangement in Chronic Myeloid Leukemia
3262. High-Resolution Genome Wide Copy Number Alteration (CNA) and Loss of Heterozigosity (LOH) Analysis in Chronic Myeloid Leukemia (CML) Shows That High and Intermediate Sokal Risk Pts (Pts) Have Multiple Losses Targeting Genes Involved in DNA Repair
3263. Imatinib Mesylate Treatment Increases Lymphoplasmocytoid Cells through SDF-1 and BMP4/7 Production in the Bone Marrow of Patients with Chronic Myelogenous Leukaemia: Relationship with Clinical/Haematological Response
3264. Loss of Growth Arrest DNA Damage 45a,b (GADD45a,b) Enhances Oncogenicity in BCR/ABL-Driven Chronic Myelogenous Leukemia
3265. Multi-Tissue, Long-Term Engraftment and Expansion of BCR-ABL-transduced Human Lin-CD34+ Cord Blood Cells in a Large Animal Model
3266. NME-2 Protein Functions as a Tumour Associated Antigen in HLA-A2+ Cells and Is Over Expressed in CML Via a Bcr/Abl Dependent Post Transcriptional Mechanism
3267. Patients with Imatinib Resistance Harbour Low Level Mutations of the BCR-ABL Kinase Domain Predominantly in the CD34+ Cells
3268. ROS-Induced DNA Damage Causing Genomic Instability in CML Stem and/or Progenitor Cells and in Quiescent and/or Proliferating Cells: Role of Mitochondrial Respiratory Chain Complex III
3269. Significance of Minimal Residual Disease in Patients with Chronic Myeloid Leukemia After Allogeneic Hematopoietic Stem Cell Transplantation
3270. Similar Patterns of Chromosome Abnormalities in CML Occur in Addition to the Philadelphia Translocation with or without Tyrosine Kinase Inhibitor Treatment
3271. Stat5 Is Essential for BCR-ABL-Transformed Chronic Myeloid Leukemia (CML) Associated with Increased CCN3 Gene Expression
3272. Targeting BCR/ABL-RAD51 Interaction to Prevent Unfaithful Homeologous Recombination Repair
3273. Targeting Isoprenylcysteine Carboxyl Methyltransferase to Overcome Resistance and Improve Responses in Chronic Myeloid Leukemia
3274. The B Cell Mutator AID Promotes B Lymphoid Blast Crisis and Drug-Resistance in Chronic Myeloid Leukemia
3275. The Broad Spectrum Bcl-2 Inhibitor Apogossypol Induces Apoptosis and Differentiation of Blast Crisis Chronic Myeloid Leukemia Stem Cells
3276. The Oncogenic Role of Tumor Suppressor Protein p27 in Ph+ Chronic Myeloid Leukemia
3277. Treatment with mTOR Inhibitor, Everolimus (RAD001) Overcomes Resistance to Imatinib in Ph-Leukemia Quiescent or T315I-Mutated Cells
3278. Ubiquitin-Mediated Binding and Stabilization of -Catenin by p210 BCR/ABL

12 Posters: Therapy I

http://ash.confex.com/ash/2009/webprogram/Session2092.html
1107. Three Novel Alternative Splicing Mutations in BCR-ABL1 detected in CML Patients with Resistance to Kinase Inhibitors
1108. Late Development of Cytogenetic Abnormalities in Ph Negative Cells of Chronic Myeloid Leukemia Patients Treated with Imatinib
1109. Outcome and Prognosis of 1955 Patients with Chronic Myeloid Leukemia: First Results of the CML-Registry of the European Treatment and Outcome Study EUTOS
1110. Comprehensive Evaluation of Time-to-Response Parameter as a Predictor of Long-Term Outcomes Following Imatinib Therapy in Chronic Phase Chronic Myeloid Leukemia
1111. Prevalence of Large Granular Lymphocyte Proliferation in Chronic Myeloid Leukemia (CML) Patients Treated with Dasatinib
1112. Relevance of the Daily Dose of Imatinib Mesylate (IM) Rather Than Its Trough Plasma Concentration for Achieving Deep Molecular Response in Patients with Chronic Myeloid Leukemia
1113. Early Optimization of Imatinib Therapy in Patients Newly Diagnosed with Chronic-Phase Chronic Myeloid Leukemia (CP-CML). A Study of the Spanish PETHEMA Group
1114. The Level of Peroxiredoxin and Catalase On Single Cell Level Is Correlated with the Positivity of Ph Chromosome in the Bone Marrow of CML Patients During Imatinib Therapy
1115. Patterns and Management of Selected Adverse Events of Adult Patients with Imatinib-Resistant or -Intolerant Chronic Myeloid Leukemia (CML) From the ENACT (Expanding Nilotinib Access in Clinical Trials) Study
1116. BCR/ABL Inhibitors Influence Phenotype and Function of Monocyte-Derived Human Dendritic Cells
1117. OCT-1 Protein Expression Detected by Flow Cytometry Provided Valuable Information in CML Patients Treated with Imatinib MesylateÃ‚ÂFHigher hOCT-1 Expression Predict Better Outcome
1118. Old Age Affects Survival but Not Response in Philadelphia Positive (Ph+) Chronic Myeloid Leukemia (CML) Patients Treated with Imatinib (IM): A Study of the GIMEMA CML WORKING PARTY
1119. Weekend Drug Holiday of Dasatinib in CML Patients Not Tolerating Standard Dosing Regimens. Reducing Toxicity with Maintained Disease Control
1120. Opportunistic Infections Are Uncommon with Dasatinib in Patients with Chronic Myeloid Leukemia in Chronic Phase (CML-CP)
1121. Hypophosphatemia During Imatinib Treatment of Newly Diagnosed Chronic Myeloid Leukemia Patients Is Associated with Better Response
1122. Resistance, Outcome and the Development of Mutations with Dasatinib in Patients with Chronic-Phase Chronic Myeloid Leukemia (CML-CP)
1123. Large Granular Lymphocyte (LGL) Expansions Comprising Oligoclonal T Cell or NK Cell Populations in Dasatinib Treated Patients Are Associated with HLA-A*0201, CMV Reactivation and Enhanced Anti-Leukemic Control
1124. The Dose and the duration of frontline therapy with Imatinib does not affect response to Dasatinib in Imatinib-Resistant or - intolerant patients with chronic myeloid leukemia (CML). Results from a real Life-Based Italian Multicenter retrospective study on 124 Patients

1125. Maintaining Imatinib ≥600 Mg Daily in the First 12 Months of Chronic Phase CML Treatment Is Associated with Superior Event-Free Survival at 5 Years
1126. International Randomized Study of Interferon Vs STI571 (IRIS) 8-Year Follow up: Sustained Survival and Low Risk for Progression or Events in Patients with Newly Diagnosed Chronic Myeloid Leukemia in Chronic Phase (CML-CP) Treated with Imatinib
1127. Comparison of Steady-State Imatinib (IM) Trough Levels, Clinical Response, and Safety Between Caucasian and Asian Patients with Chronic Lyeloid Leukemia in Chronic Phase (CML-CP) Treated with 400mg and 800mg Daily Doses of IM in the Tyrosine Kinase Inhibitor Optimization and Selectivity (TOPS) Study
1128. Dasatinib Is Well-Tolerated and Efficacious in Imatinib-Intolerant Patients with Chronic-Phase Chronic Myeloid Leukemia (CP-CML)
1129. Update On Imatinib-Resistant Chronic Myeloid Leukemia Patients in Chronic Phase (CML-CP) On Nilotinib Therapy at 24 Months: Clinical Response, Safety, and Long-Term Outcomes
1130. Response and Outcomes to Nilotinib at 24 Months in Imatinib-Resistant Chronic Myeloid Leukemia Patients in Chronic Phase (CML-CP) and Accelerated Phase (CML-AP) with and without BCR-ABL Mutations
1131. Early Dose-Escalation in Chronic Myeloid Leukaemia Patients with Low Plasma Imatinib Levels Leads to Equivalent BCR-ABL Values and Drug Levels at 6 Months to Those with Optimal Drug Levels: First Analysis From the TIDEL II Trial of De-Novo Patients Treated with 600mg Imatinib

13 Posters: Therapy II

http://ash.confex.com/ash/2009/webprogram/Session2231.html
2192. The Combination of Interferon-Alpha with Imatinib in Early Chronic Phase Chronic Myeloid Leukemia Patients Induces a Significant Improvement of the Molecular Responses in the First Two Years of Treatment: Results From Three Studies From the GIMEMA CML Working Party
2193. A Phase 2 study of the Combination of Omacetaxine and Imatinib in the Treatment of Patients with Chronic Myeloid Leukemia (CML) in Advanced Stages or After Failure to Imatinib
2194. A Phase I Study of the HDAC Inhibitor LBH589 in Combination with Imatinib for Patients with CML in Cytogenetic Remission with Residual Disease Detectable by Q-PCR
2195. Combined Chemotherapy (daunorubicin + cytarabine) and Dasatinib as Salvage Therapy of Chronic Myeloid Leukemia (CML) in Myeloid Blast Crisis, a Pilot Study
2196. Chronic Myeloid Leukemia (CML) Patients with "Suboptimal" Response to Imatinib (IM) According to European LeukemiaNet Criteria Have a Poorer Outcome with Respect to "Optimal" Responders: A GIMEMA CML WORKING PARTY Analysis
2197. Current Leukemia Free Survival After Tyrosine Kinase Inhibitor (TKI) for Chronic Myeloid Leukemia (CML): A New Method That Accounts for Restoring Response with Sequential TKI
2198. Dasatinib Once-Daily Dose Regimen Provides Robust Anti-Leukemic Activity While Avoiding Suppression of T Cell Activation
2199. Imatinib Long Term Effects (ILTE) Study: An International Study to Evaluate Long-Term Effects in CML Patients
2200. Imatinib Mesylate Induced Cardiotoxicity: a Cardiac Progenitor Cell Disease?
2201. Impact of Prior Therapy and Suboptimal Response to Imatinib On the Efficacy and Safety of Nilotinib Among 1,422 Patients with Imatinib-Resistant or –Intolerant Chronic Myeloid Leukemia (CML) in Chronic Phase (CP): Sub-Analyses of the ENACT (Expanding Nilotinib Access in Clinical Trials) Study
2202. In Vitro and In Vivo Study of Synergistic Effect of Imatinib and Simvastatin in Chronic Myelogenous Leukemia
2203. Kinase Domain Mutations and Responses to Dose Escalation in Chronic Myeloid Leukemia Resistant to Standard Dose Imatinib Mesylate
2204. Molecular and Functional Analysis of Large Granular Lymphocyte Expansions in Chronic Myelogenous Leukemia Patients Undergoing Tyrosine Kinase Inhibitor Therapy
2205. Nilotinib 800 Mg Daily as Frontline Therapy of Ph + Chronic Myeloid Leukemia: Dose Delivered and Safety Profile for the GIMEMA CML Working Party
2206. Nilotinib-Associated Molecular Responses Achieved in Chronic Myeloid Leukemia in Chronic Phase (CML-CP) Patients with a Suboptimal Molecular Response to Imatinib
2207. Persisting Chronic Myeloid Leukemia Stem and Progenitor Cells From Patients in Major Molecular Remission Under Imatinib Are Characterized by Low BCR/ABL Expression
2208. Polymorphisms in the Multidrug Resistance Gene MDR1 (ABCB1) Predict for Molecular Resistance in Patients with Newly Diagnosed Chronic Myeloid Leukemia (CML) Receiving High-Dose Imatinib
2209. Predictors of Treatment Non-Adherence in Patients Treated with Imatinib Mesylate for Chronic Myeloid Leukemia
2210. Prognostic Factors in Treatment of Chronic Myeloid Leukemia with Tyrosine Kinase Inhibitors- Single Center Experience
2211. Real-Life Analysis of Dasatinib in Chronic Phase CML Patients Aged > 60 Years Resistant/Intolerant to Imatinib
2212. Reduced Expression Level of SHP1 Gives An Additive Survival Advantage to the Ph+ Cells of Chronic Myeloid Leukemia (CML) Patients and Provides a Novel Pretreatment Predictor of Major Molecular Response Achievement in CML Patients
2213. Steady-State Imatinib Trough Levels as Well as Dose Interruptions Are Associated with Clinical Response (CCyR and MMR) and Adverse Events (AEs) in Patients with Chronic Myeloid Leukemia (CML) Receiving IM as Frontline Therapy
2214. T-Cell and B-Cell Responses After Vaccination against Influenza Virus and Pneumococcus in Chronic Phase CML Patients Treated with Tyrosine Kinase Inhibitors
2215. The Gene RUNX1 and Its Possible Relation with the Alteration of Granulocytes Cells and with the Progression of Chronic Myeloid Leukemia
2216. The Plateau of BCR-ABL Transcript Level ≥0.1% May Select CML Patients in Complete Cytogenetic Remission for Mutation Analysis

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http://ash.confex.com/ash/2009/webprogram/Session2232.html
3279. T/NK Lymphocytosis in CML Ph+ Patients During Dasatinib Therapy
3280. A Randomized Phase II Study Comparing Imatinib and the Combination of Imatinib and Pegylated Interferon Alpha-2b in Newly Diagnosed Non-High Risk Chronic Myeloid Leukemia (CML) Patients in Complete Hematological Remission After Imatinib Induction Therapy
3281. Allogeneic Hematopoietic Stem Cell Transplantation (HSCT) with a Related Donor in Chronic Myeloid Leukemia (CML): An Explanation for Fast Improvement of Survival in Two Consecutive German CML Studies
3282. Analysis of Molecular Data and the Emergence of Mutations for Chronic-Phase Chronic Myelogenous Leukemia (CML-CP) Patients Treated with Dasatinib After Imatinib Failure
3283. Association Between Imatinib (IM) Transporters and Metabolizing Enzymes Genotype and Response in Newly Diagnosed Chronic Myeloid Leukemia (CML) Patients (Pts) Is Influenced by Ethnicity

3284. Candidate Pathway Approach of Single Nucleotide Polymorphism On Imatinib Transport/Metabolism Pathway and DNA Repair Enzyme Pathway Associated with Response and Resistance to Imatinib Therapy in Chronic Myeloid Leukemia
3285. Differential Effects of Imatinib and Dasatinib On Immune Effector Cells in Patients with Chronic Myeloid Leukemia (CML)
3286. Efficacy and Safety of Nilotinib in Elderly Patients with Imatinib-Resistant or –Intolerant Chronic Myeloid Leukemia (CML) in Chronic Phase (CP): A Sub-Analysis of the ENACT (Expanding Nilotinib Access in Clinical Trials) Study
3287. Evolution of Blast Crisis (BC) in Chronic Myeloid Leukemia (CML) in the Imatinib-Era: A Rare Event with High Proportions of Low Risk Patients and of Early Bc; Need for Rapid Detection. Results of the German CML Study IV
3288. Front-Line Imatinib Mesylate (IM) in Patients with Newly Diagnosed Accelerated Phase (AP)-Chronic Myeloid Leukemia (CML), a Study From the FILMC Group (France Intergroupe Leucemie Myeloide Chronique)
3289. Impact of Imatinib Dose Escalation in Chronic Myeloid Leukemia Patients in Chronic Phase with Sub-Optimal Response or Failure with Imatinib 400 Mg
3290. Long Term Adherence to Imatinib Therapy Is the Critical Factor for Achieving Molecular Responses in Chronic Myeloid Leukemia Patients
3291. Low Risk of Infectious Events in Patients (Pts) with Chronic Myeloid Leukemia (CML) in Chronic Phase (CP) Treated with Dasatinib
3292. Molecular Response at 3 Months On Nilotinib Therapy Predicts Response and Long-Term Outcomes in Patients with Imatinib-Resistant or -Intolerant Chronic Myeloid Leukemia in Chronic Phase (CML-CP)
3293. Molecular Response, Efficacy and Safety Analysis of 168 Adult French Patients with Chronic Myeloid Leukemia (CML) in Chronic Phase (CP) From the ENACT (Expanding Nilotinib Access in Clinical Trials) Study
3294. Nilotinib 300 Mg Twice Daily Is Effective and Well Tolerated as First Line Treatment of Ph-Positive Chronic Myeloid Leukemia in Chronic Phase: Preliminary Results of the ICORG 0802 Phase 2 Study
3295. Nilotinib Responses and Tolerability Confirmed in North American Patients with Chronic Myeloid Leukemia (CML) From ENACT (Expanding Nilotinib Access in Clinical Trials)
3296. Predictors of Long-Term Cytogenetic Response Following Dasatinib Therapy of Patients with Chronic-Phase Chronic Myeloid Leukemia (CML-CP)
3297. Prior Response to Imatinib Predicts Response to Second Line Treatment with Nilotinib in CML Patients Resistant or Intolerant to Imatinib
3298. Prognostic Factors for Progression-Free Survival in Patients with Imatinib-Resistant or -Intolerant Chronic Myeloid Leukemia in Chronic Phase (CML-CP) Treated with Nilotinib Based On 24 Month Data
3299. Prognostic Significances of ABCG-2 and hOCT-1 Gene Expression in Chronic Phase Chronic Myeloid Leukemia Patients Treated with Imatinib Mesylate
3300. Quantitative PCR Using a Nanofluidic Platform to Quantify Bcr-Abl mRNA in Patients Who Are Negative by Routine Quantitative PCR Testing
3301. Relationship of WT1 Gene Expression to Disease Progression and Imatinib Responsiveness in Chronic Myelocytic Leukaemia Patients
3302. Results of Imatinib Dose Escalation After 36 Months of Follow-up in Chronic Myeloid Leukemia Patients with Failure or Sub-Optimal Response According to 2006 EuropeanLeukemia Net (ELN) Criteria
3303. WP1130 Inhibits Signaling through BCR-ABL Ubiquitination and Cytoplasmic to Aggresome Trafficking to Induce Apoptosis of CML Cells