- **To view the original ASH abstracts go to: https://ash.confex.com/ash/2014/webprogram/start.html**
- Look for topics 631 (mostly biology) and 632 (mostly clinical) and you'll find the vast majority of the CML abstracts. This year there were 30 oral presentations and 127 posters. I've included in this document all 30 of the oral presenation abstracts and a listing of all the posters.
- For the poster abstracts, clicking on the section heading title should take you to those abstracts on the web.

1 Highlights

Five CML themes in this years meeting:

- **Updates on clinical trials** including cardiovascular risk
- **Stopping treatment** and factors associated with recurrence
- **New technologies**, particularly next generation sequencing and digital PCR
- Some useful **long term observations** from registries
- Lots of new data on **CML in children** – more than I recall seeing at any previous meeting

1. Updates on clinical trials

There were lots of updates on clinical trials and there are **comparative data** between imatinib and **nilotinib** (ENESTnd, imatinib vs nilotinib, [4541]) and **dasatinib** (Dasision, imatinib vs dasatinib, similar to SPIRIT 2 but with 1 yr rather than 5 yr endpoint) [154]. There were reports on a dasatinib phase II front line study [4565], 7 year follow up of one of the original dasatinib studies [520], **bosutinib** [4559] and the French SPIRIT trial (I400 vs I600 vs I+**IFN**) [1793] as well as imatinib vs ponatinib (EPIC [519]). There were a number of abstracts about **ponatinib** [518, 4552, 4558] including front line use [519, 4535] and an update on the PACE trial [3135]. This drug (as well as nilotinib) has struggled for the last year because of concerns over cardiovascular events but it is possibly the most effective TKI and the key to getting it right might be dose [4546, 3153]. We'll be evaluating the selective use of dose-optimised ponatinib in the NRCI **SPIRIT 3** trial in patients who are not responding optimally to first line treatment.

I was delighted to be able to present the NCRI **SPIRIT 2** study for the first time [517]. This is the largest dasatinib trial (n=814) to date. MR3 (major molecular response) rate at one year is 58.4% with dasatinib and 43.1% with imatinib ($p<0.001$) but there is no difference in progression or survival.

[1] This review is provided free of charge as a service to the CML community and may be used, abused, distributed, shared or deleted without trace as you see fit without having to seek the permission of the author. Views expressed are those of the author alone; this work has not been commissioned/paid for by any organization, pharma company or otherwise and represents, for good or ill, the independent views of Prof O'Brien. The author is always keen to improve and any comments, good or bad, would be gratefully received (stephen.o'brien@ncl.ac.uk). All cited data are freely available in the public domain.

This is a **common theme** with other studies. To date all the 2nd generation compared to imatinib studies have shown **higher molecular response rates** but **no differerence in overall survival**. The 5 TKIs all have increasingly well defined side effect profiles: dasatinib – pleural effusion (22% in SPIRIT 2) but no cardiovascular signal (see below); nilotinib and ponatinib – cardiovascular events; bosutinib – GI toxicity common but no apparent cardiovascular problems.

Imatinib therefore remains a very **reasonable option for first line therapy** especially as it will come off patent in 2016 in many countries. Indeed it already is **off patent** in Canada for example where competition from at least two competitors (Teva and Apotex) is driving the price down. This is a welcome development for struggling health services that I think will lead to more rather than less imatinib use across the world.

So what's the point of the newer TKIs? They clearly have a role a) in patients not responding well to imatinib; b) in patients for whom deep response and stopping treatment is important. So it's not 'one size fits all' and the key issue therefore is how to figure out which patients need the more potent (and expensive) drugs and when to intervene. It's increaslgly apparent that there is a **balance to be struck** between efficacy and toxicity, especially cardiovascular toxicity.

Using **early PCR** values to predict response remains topical [4561] with some suggesting that response after only 1 month on treatment is predictive [816]. We've heard about the importance of 'less than 10% at 3 months' for some time now but it's emerging that perhaps the **slope of the early response** is more useful [156, 816, 3148]. So going from 90% to 11% in the first 3 months might be 'better' than going from say 13% to 9%. Most such analyses have been applied to imatinib but the same seems to hold true for dasatinib [1795]. **Prognosis** may be determined not just by early response to treatment but perhaps by TGF-α and IL6 levels [1788], as well as polymorphisms in BIM (BCL2L11) [1797]. We're gradually getting better at identifying patients for whom a change of therapy might be a good idea. Sequencing might help further (see later).

There were updates on various aspects of **cardiovascular risk** and TKIs: it's still not well understood. CV risk seems to mainly be a concern with nilotinib and ponatinib and there's some reassuing evidence that there is not such a concern if patients don't have prior CV risk factors [1811]. Mouse and human data on **mechanism** with nilotinib were presented [1800]. Development of insulin resistance in some patients on nilotinib might be important [1813] and more light is being shed on potential mechanism of CV events with ponatinib [1783] but I think it's fair to say this whole area is still poorly understood. Dasatinib (and bosutinib) appears to carry less CV risk [4534] – helpful to know when selecting the right treatment for individual patients. The *SCORE* chart can identify patients on nilotinib at risk [4545] and homocysteine levels might be important [3136]. Useful case series/registry data also contribute to our understanding [3234, 3147]. In then UK we'll be using the **QRISK2** score to evaluate cardiovascular risk in **SPIRIT 3**.

Here's an interesting observation: many CML patients will end up on a statin and there's some data (albeit retrospective and with no PK) that **patients on statins** have higher MMR rates [1804]. That might be due to CYP3A4 interactions pushing imatinb levels up but who knows, maybe statins have an anti-leukaemic effect. Not convinced….

2. Stopping treatment and factors associated with recurrence

Stopping continues to be very topical. The EuroSKI study was presented for the first time [151]. This is similar to the original STIM study and by October of this year, 648 patients had been registered and 200 were evaluable after 6 months. Patients had to have been on a TKI for at least 3 years and been in MR4 for at least a year. So far the 18 month KM probability of relapse-free survival is 55% (95% CI 47%-61%) which is higher than the original STIM study. In contrast the ISAV study [813] seemed to show a lower rate of durable *complete* molecular remission. 112 patients were enrolled in this study: Only 15% maintained CMR, 48% relapsed although the remaining 37% didn't go above MR3 and some stayed at that low level for quite some time. There was a higher rate of relapse amongst young patients - not known why.

Results from **other imatinib stopping studies** are coming through: ENESTnext [1796], SENSOR [1815], Gimema study [4532], Korean studies [1816, 3155, 4553] and rates of successful stopping vary. There was an update from the French **stopping 2nd generation TKI** study – the only one of it's kind so far and with still a relatively small number of patients, n=52 [811]. KM probability of treatment-free survival in MMR (CMR data not presented) at 24 months is 57.4% (95%CI 43.9-70.9). There was an interesting abstract about **patients' attitudes** toward stopping in Hungary [4547]. Most, but not all, are keen.

There were a few abstracts looking at **factors associated with molecular relapse** following stopping. It's still not very clear but there are suggestions that it might be related to: polymorphisms in BIM (BCL2L11) [1797]; low number and impaired function of NK cells [812]; age and results of dPCR [813] (more later…). Some, but not all,

studies suggest that duration of prior TKI therapy is also important.

As more studies report it's becoming clearer that, following stopping, **some patients can relapse quite late**, say after a few years, so ongoing monitoring remains important. Reassuringly in all of these studies no patients have gone into blast crisis and patients regain molecular response if therapy is restarted. The INTERIM study is a bit different and is looking at ***intermittent*** **imatinib** in elderly patients: giving less seems to be OK in older patients [1794].

There seem to be **two emerging schools of thought**: there are those (including most TKI companies) who believe that **stopping is the highest priority** and deep molecular responses important as a route to 'cure' (however you might wish to define that). Others accept that **having a small amount of residual disease is acceptable** if patients live a normal life span and have few, preferably no, short or long term side effects. There's a (financial and wellbeing/utility) price to pay in achieving very deep molecular response and this may not be worth it for many CML patients especially those who are older and have significant co-morbidities. The approach of reducing dose at the point of MMR is for example being evaluated in the UK **DESTINY** study at present and we may have data at next year's ASH. Reducing as a prelude to stop will also be part of **SPIRIT 3**.

3. New technologies, particularly next generation sequencing and digital PCR

Next generation sequencing (NGS) is really starting to fly but it's still quite expensive (although rapidly coming down in price) and produces a ferocious amount of data requiring powerful bioinformatics. I'm a fan: I think patient sequencing reports will be as common as morphology and flow cytometry reports quite soon [399]. Technology platforms are jostling for position but Illumina seems to be in the lead and they recently announced the first '$1,000 whole genome' (although the kit to do it cost $10M…).

Most reports at ASH related to targeted sequencing, mainly looking at ABL kinase domain mutations (there are over 100 reported now) [815, 1810, 4525, 4531]. **T315I** remains the most important. Mutatations associated with resistance are also being found in **non-BCR-ABL genes** [4514, 4516]. NGS can also identify **DNA methylation patterns** indicative of disease progression [4526]. Whilst these targeted approaches are useful what is potentially very exciting are whole exome (~1% of the genome) or whole genome sequencing approaches. These analyses may allow us to better understand the genomic basis of response, resistance, progression and toxicity in due course although the data analysis and interpretation will be an enormous task. We're planning some large scale sequencing analyses in SPIRIT 3.

I like the look of **digital PCR** [813, 1817, 1792]. The are 4 technology platforms at present – 2 chip-based and 2 droplet-based. Is this the 'iPhone of PCR technology'? dPCR potentially offers greater sensitivity [4540] and reduced cost but the runs are generally slower so throughput reduced. Because **absolute numbers of molecules** are measured, this approach in theory does away with the need for the 'international scale' but as correlative data with conventional qPCR are somewhat lacking at present it's probably not ready for prime time just yet. But I personally think it's the future. Cepheid PCR technology also looks interesting [1809] but struggles at lower levels it seems – tech updates in progress.

At the opposite end of the tech spectrum it seems you can ship samples of **blood dropped on paper** across the world [4566] and still be able to do reliable PCR testing. This very practical piece of work could greatly extend the availability of PCR testing across the globe.

4. Some useful long term registry observations

Imatinib was first given to a CML in 1999 so we now have 15 years of experience with TKIs. There was an update on the EUTOS registry [3160] as well as data on the incidence of CML across Europe [3145]. Survival with imatinib is now not much different to the normal population [1801] and increasingly we are therefore having to advise patients about planning their families. An update on the Italian Gimema registry of conception/pregnancy [1806] was therefore useful. We sometimes get asked by patients what is known about the effects of TKIs on fertility: I'm now better equipped to answer if those patients are mice… [1799].

The Swedish registration system is impressive: all patients with cancer must be registered by law (maybe we should do that in the UK?) so there are pretty reliable data available. As well as contributing to our understanding of cardiovascular risk [3134] there was an excellent presentation on the development of second malignancies in 887 patients with CML diagnosed between 2002 and 2011 – 3,293 'person years at risk' [154]. There's a 50% higher prevalence of second malignancies in CML patients compared to the 'normal' population – standard incidence ratio (SIR) is 1.5 observed/expected (95% CI 1.13-1.99). Makes you wonder if we should be doing more screeing.

5. Data on CML in children

Although rare, there seemed to be a lot more about CML and TKIs in children this year. There were very informative **surveys of outcome**, mainly on imatinib [1803, 521] as well as data on **predicting response** [4549] and the impact of **additional cytogenetic abnormalities** [3137]. Data were also presented on decisions taken in **children who failed imatinib** [1798]. Although imatinib is producing **very good outcomes** [1812] it does seem to be associated with **growth retardation** in pre-pubescent children although not so much in older teenagers [522]. Combining **imatinib with RIC** transplant is also being evaluated [4568]. People generally seem to be favouring ongoing TKI therapy rather than elective transplant but what's unknown is whether these young patients could/should remain on TKIs for decades potentially. There are no paediatric TKI stopping studies yet.

6. And in other news…

A few **new drugs** were surfacing, all very early and I'm still not convinced there's room for more drugs in CML but here's a few: **ABL001** is a new drug that works against T315I [398], like ponatinib. There's lots of interest in **ABT199** (a Bcl-2 inhibitor) in lymphoid malignancy and now there are some data to suggest that it may be useful in combination with TKIs to eradicate CML stem cells [512]. There was a poster on combination of dasatinib with **BMS-833923** (a smoothened inhibitor) [4539]. I've never heard of **pyrvinium** before but this anti-helminthic drug appears to be effective in blast phase CML (not in patients as yet) by inhibiting mitochondrial respiration [514]. And to round up there were data on **copanlisib** (PI3K inhibitor) [3127]; **synthetic anti-IL3 receptor antibodies** [4521]; **chaetocin** (a non-specific histone lysine methyltransferase inhibitor) [4517]; **ethacrynic acid** derivatives [4508] and **BGB324** (an AXL inhibitor) [4512].

CAR-T cells [966] continue to look very exciting in refractory acute leukaemia [380] and other indications: lymphoma [3087]; CLL [1982]; Hodgkins [806]. **We're not seeing use in CML yet**: there's isn't such an obvious antigenic target and this approach is pretty hard work. Most patients develop a cytokine release syndrome [1983, 2296] that often leads to a trip to the ITU. But I suspect this will be refined given time and I think this is one of the most successful immune therapies to date.

All in all a pretty good ASH: really exciting new technologies coming through and maturing clinical data that will in time help us refine the balance between risk and benefit that we need to strike to optimise treatment for our patients.

2 Outcomes With TKI Therapy [151-156]

[151] Interim Analysis of a Pan European Stop Tyrosine Kinase Inhibitor Trial in Chronic Myeloid Leukemia: The EURO-SKI study. *Mahon.* **Background:** The tyrosine kinase inhibitors (TKIs) have dramatically changed the natural history of chronic myeloid leukemia (CML) leading to significant improvement in clinical outcome and survival rates. The option of treatment cessation has recently become of utmost importance. Indeed, prospective trials suggest that imatinib therapy may be safely and successfully discontinued in CML pts with deep and sustained molecular responses (Mahon Lancet Oncol 2010, Ross Blood 2013). The major aim of the EURO-SKI study (European Leukemia Net Stop TKI study) was to define prognostic markers to increase the rate of patients in durable deep MR after stopping TKI. Further aims were the evaluation of harmonized methods of molecular monitoring, assessment of quality of life, and calculation of saved treatment costs per country. **Methods:** Adult CML patients in chronic phase CML on TKI treatment in confirmed deep molecular response (MR4, BCR-ABL <0.01%) for at least one year (>4 log reduction on TKI therapy for >12 months confirmed by three consecutive PCR tests) and under TKI treatment for at least 3 years were eligible. MR4confirmation was performed in a standardized laboratory (n=6). Primary endpoint was the assessment of the duration of MR (defined by continuous MMR) after stopping TKI. Patients (pts) after a prior TKI failure were excluded. According to protocol, an interim analysis was planned after 200 patients with eligible molecular results at month (mo) 6 were available to test the null hypothesis that relapse-free survival at 6 mo is less or equal 40%. **Results:** From June 2012 to June 2014, 498 CML pts in chronic phase from 10 countries were enrolled and included in the trial. From June 2012 to July 2013, 254 pts from 8 countries were registered; 54 were excluded (consent withdrawal n=1, protocol violation n=1, not eligible n=34, restart of TKI without relapse n=4, atypical or unknown transcript n=6, missing data n=8). Of the eligible 200 pts, 41.5% were female. Median age at diagnosis was 53.3 years (range, 13.8 to 85.5). In assessable pts 8.7% and 18.2 % were high-risk according to EUTOS and Sokal Scores. 103 pts were treated prior to the start TKI therapy, mostly with hydroxyurea or interferon. 1st-line TKI was imatinib in 97%, dasatinib in 1.5%, and nilotinib in 1.5% of pts. Twenty-four pts switched to second-line TKI therapy due to intolerance, 16 to dasatinib, 2 to imatinib, and 6 to nilotinib. The median time from diagnosis of CML to TKI cessation was 8 years (range, 3-19 years). TKI treatment duration was less than 5 years in 16%, 5-8 years in 36% and > 8 years in 48% of pts. Median duration of TKI treatment was 8 years (range, 3-12.6 years) and median duration of MR4 before TKI cessation was 5.4 years (range, 1-11.7 years). MR4duration was less than 2 years in 8%, 2-5 years in 37%, 5-8 years in 39% and >8 years in 16% of pts. For all eligible pts, a standardized European laboratory confirmed MR4 assessment. Since 123 of the 200 pts (61.5%, 95% CI: [54.4%; 68.3%]) remained without relapse the first 6 mo, the null hypothesis could be discarded (p<0.0001). Recurrence of CML, defined as loss of MMR, was observed in 43/92 pts (47%) treated <8 years, as compared to 23/87 pts (26%) treated for >8 years (p= 0.005). So far, there was a trend for prognostic significance of MR4 duration: 33/71 pts with MR4 <5 years (46%) lost MMR within 6 mo as compared to 28/87 pts (32%) with MR4duration >5 years (p=0.07). No significant difference was observed for relapse within 6 mo according to depth of molecular response at discontinuation (MR4 vs MR4.5 vs MR5). TKI cessation was a safe procedure but a substantial proportion of pts reported transitory musculoskeletal pain starting within weeks after imatinib discontinuation. The phenomenon was described in 30% of Swedish patients as a “TKI withdrawal syndrome” (Richter JCO 2014). Taking into account the cost of imatinib in Europe and time without treatment in the total study population at the most recent analysis, total savings for the community within the EURO-SKI trial were estimated at 7 million Euros. **Conclusion:** Employing a standardized molecular testing for patient selection within a TKI cessation trial in CML the chance to stay in treatment-free remission could be higher than previously reported. The EURO-SKI trial will further elucidate the prognostic factors but the preliminary results confirm (as reported in the STIM Study) the prognostic impact of the duration of TKI therapy before stopping.

[152] Final Study Results of the Phase 3 Dasatinib Versus Imatinib in Newly Diagnosed Chronic Myeloid Leukemia in Chronic Phase (CML-CP) Trial (DASISION, CA180-056). *Cortes.* **Background:** The randomized, phase 3 DASISION trial demonstrated improved efficacy with dasatinib compared with imatinib in treatment-naïve CML-CP patients (pts). Dasatinib was also well tolerated, and demonstrated a faster response at 3 months. Here, we report the results of the final, 5-year analysis of DASISION. **Methods:** Pts with newly diagnosed CML-CP were randomized to receive dasatinib 100 mg once daily (n=259) or imatinib 400 mg once daily (n=260) as previously reported. The primary endpoint was confirmed complete cytogenetic response (cCCyR) by 12 months. Long-term efficacy and safety data from pts with the predefined minimum 5 years of study treatment are presented. **Results:** Sixty-one percent of dasatinib-treated pts and 63% of imatinib-treated pts were still on their initial study therapy at study end. Cytogenetic and molecular response rates continued to be higher for dasatinib compared with imatinib (intent-to-treat population). Specifically, the rate of cCCyR by 5 years was higher with dasatinib versus imatinib (83% vs 78%, *P*=.187), as were the rates of major molecular response (MMR; BCR-ABL ≤0.1%; 76% vs 64%, *P*=.002) and MR4.5 (BCR-ABL ≤0.0032% IS; 42% vs 33%, *P*=.025) by 5 years. Time to cCCyR (hazard ratio [95% confidence interval]=1.46

[1.20–1.77], *P*=.0001) and MMR (hazard ratio [95% confidence interval]=1.54 [1.25–1.89], *P*<.0001) in all randomized pts were faster with dasatinib (Figure 1). Transformations to both accelerated (AP) and blast phase (BP) CML were reported on study or after discontinuation with fewer cases overall for dasatinib (n=12/259; 4.6%) compared with imatinib (n=19/260; 7.3%). Five-year progression-free survival (PFS) and overall survival (OS) rates were similar across treatment arms (PFS: 85% [dasatinib], 86% [imatinib]; OS: 91% [dasatinib], 90% [imatinib]). A higher proportion of pts on dasatinib achieved BCR-ABL ≤10% at 3 months (84%) compared with those on imatinib (64%). For pts who achieved BCR-ABL ≤10% versus >10% at 3 months, improved PFS, OS, and lower rates of transformation to AP/BP have been previously reported and were maintained at 5 years for dasatinib (PFS: 89% vs 72%, *P*=.0014; OS: 94% vs 81%, *P*=.0028; transformation n=6/198 [3%] vs n=5/37 [14%]) and imatinib (PFS: 93% vs 72%, *P*<.0001; OS: 95% vs 81%, *P*=.0003; transformation: n=5/154 [3%] vs n=13/85 [15%]). Between 4 and 5 years, the number of mutations increased slightly in dasatinib-treated pts (12 pts at 4 years; 15 pts at 5 years), and the spectrum remained the same. No new, unexpected safety events were identified in either treatment arm at 5 years. However, the total incidence of pleural effusion continued to increase each year in dasatinib-treated pts (29% overall). Most cases of pleural effusion were grade 1/2 (n=67/74), and the median time to first grade 1/2 pleural effusion was 114 weeks (range, 4–299 weeks). Discontinuation of dasatinib due to pleural effusion occurred in only 15 pts (6% overall; 20% of pts who experienced a pleural effusion). Arterial ischemic events overall were not common, occurring in 12 pts (5%) on dasatinib and 6 pts (2%) on imatinib. Cardiovascular (CV) ischemic events and transient ischemic attack were reported in 10 and 2 dasatinib-treated pts, respectively. CV ischemic and peripheral arterial occlusive events were reported in 4 and 2 imatinib-treated pts, respectively. Of the pts with overall arterial ischemic events, 8 pts on dasatinib and 3 pts on imatinib had a history and/or risk factors for atherosclerosis. Fourteen dasatinib-treated pts experienced pulmonary hypertension by 2D echocardiogram, with right heart catheterization (RHC) performed in 1; 6 discontinued therapy. No pts were diagnosed with World Health Organization Group 1 pulmonary arterial hypertension (confirmed by RHC). **Conclusion:** At 5 years, dasatinib 100 mg once daily has demonstrated superior outcome compared to imatinib 400 mg once daily as initial therapy for CML. This is manifested by a faster time to cytogenetic and molecular responses, with more pts achieving BCR-ABL ≤10% at 3 months, sustained higher cumulative rates of response, and a lower rate of transformation. The 5-year rates of PFS and OS were equal in both arms. After 5 years, no new safety signals have been reported. These consistent results suggest that dasatinib offers meaningful advantages for pts with newly diagnosed CML-CP and remains a standard of care in this setting.

[153] Survival and Prognosis in Patients with First-Line Imatinib Treatment Under Particular Consideration of Death Due to Chronic Myeloid Leukemia. *Pfirrmann.* ***Introduction***: The IN-study section of the European Treatment and Outcome Study (EUTOS) registry comprises data on imatinib-treated patients with chronic myeloid leukemia (CML) who were enrolled between 2002 and 2006 in prospective, controlled clinical trials. Of those, 2290 adult patients had Philadelphia chromosome-positive chronic-phase (CP) CML and were eligible for analysis and prognosis of long-term survival. Improved survival increased the percentage of deaths not related to CML. While adjusting for this, our analyses put death due to CML into focus. ***Aims***: Based on the observed survival in our patient sample and on survival in matched population data, relative survival (RS) probabilities attributable to the excess hazard of CML should be calculated for the 2290 patients. These results were to be opposed to cumulative incidences of mortality (CIM) when only death due to CML is considered as an event and all other causes of death as competing risks. The ability to discriminate CIM of dying from CML should be assessed for the established prognostic models Sokal, Euro, and EUTOS score and a possibly identified new model. Candidate factors were age, sex, spleen enlargement, hemoglobin, platelets, leukocytes, and percentages of blasts, eosinophils, and basophils in peripheral blood. ***Methods****:*Survival time was calculated from the date of start of treatment to death or to the latest follow-up date. Survival was censored at the time of allogeneic stem cell transplantation in first CP. As “death due to CML”, only death after recorded disease progression was regarded. Progression was given by observation of accelerated phase or blast crisis, both defined in accordance with the recommendations of the ELN (Baccarani et al Blood 2013). RS probabilities were calculated by the method of Pohar-Perme (Comput Biol Med 2007) and CIM by the cumulative incidence function. Population data was downloaded from the Human Mortality Database (www.mortality.org). All prognostic factors were measured at baseline and the influence on CIM due to CML was estimated by the Fine and Gray (FG) model. Level of significance was 0.05. ***Results****:*The 2290 patients came from study groups in Germany, France, Italy, Spain, the Netherlands, and the Nordic study group and had a median observation time of 6.4 years. Observed 8-year overall survival probability was 89% [95% confidence interval (CI): 87-90%] and 8-year RS probability 96% [95% CI: 93-97%]. Cause of death was due to CML in 92 of 208 cases (44%), unrelated to CML in 104 (50%), and unknown in 12 cases (6%). Eight-year CIM were 4% [CI: 4-5%] for causes of death due to CML and 7% [CI: 6-8%] for causes of death due to other reasons, including the unknown causes where no progression prior to death was observed. From low to high risk groups, in 2205 evaluable patients, the Sokal score resulted in 8-year CIM of 3% [95% CI: 2-4%], 4% [95% CI: 3-6%], and 7% [n=499, 95% CI: 5-10%] and the Euro score in 8-year CIM of 4% [95% CI: 3-5%], 3% [95% CI: 2-4%], and 12% [n=222, 95% CI: 8-17%]. The EUTOS score suggested two

groups with 8-year CIM of 4% [95% CI: 3-5%] and 9% [n=232, 95% CI: 5-13%]. Higher age, more blasts, a bigger spleen size enlargement, and low platelet counts significantly increased the CIM of dying from CML. The four factors were combined in a new prognostic model. Here, 8-year CIM were 2% [n=1349, 95% CI: 1-3%], 6% [n=596, 95% CI: 4-8%], and 11% [n=260, 95% CI: 8-16%]. ***Conclusions***: An 8-year RS probability of 96% corresponded to an estimated 4% probability of dying due to CML which actually was the same result as the one calculated for the CIM. However, while for the first method, access to matched population data is necessary but no knowledge on the cause of death, in the second case, investigators need to assess whether an individual died from CML or not. Using the "progression prerequisite", the FG model was most likely only based on "real" cases of death due to CML. As causes of death without prior progression, like infection or treatment-related toxicities, might well be attributable to CML, the CIM of death due to CML were supposedly underestimated. For assessment of comparability between patient samples, prognostic models built from baseline variables remain important. In comparison to other scores, only the new model identified three risk groups with pairwise significantly different CIM and led to the largest high-risk group with an 8-year CIM above 10%. Independent data for further comparisons are collected.

[154] Second Malignancies Following Treatment of Chronic Myeloid Leukemia in the Tyrosine Kinase Inhibitor Era. *Själander.* **Background:** Since continuous treatment with tyrosine kinase inhibitors (TKIs) has dramatically improved the survival of patients with chronic myeloid leukemia (CML), it is of interest to examine the possible risk of long-term adverse events. Previous studies have presented conflicting results regarding risk of second malignancies. Our aim was to examine the development of second malignancies (except acute myeloid or lymphoblastic leukemia, myelodysplastic syndromes or non-melanoma skin cancer) in CML chronic phase patients diagnosed after the introduction of TKI treatment. **Materials and methods:** We studied the development of second malignancies in 868 patients diagnosed with CML in chronic phase 2002 to 2011 using the Swedish CML register, cross-linked to the Swedish Cancer register. Each patient was followed from the time of CML diagnosis until death from any cause, date of allogeneic hematopoietic stem cell transplantation (SCT) or end of study on December 31, 2011, whichever came first. SCT was used as an endpoint because of the well established increased risk of second malignancies after this procedure. Standardized Incidence Ratios (SIR) were calculated to assess the risk of a second malignancy by dividing the number of observed second malignancies with the number of expected malignancies in the Swedish population, using data from the Swedish Cancer Register. The expected numbers of malignancies were determined by dividing the CML population according to 5-year age groups, sex, region of residence (6 regions) and calendar year. The number of person-years in each stratum was multiplied with the incidence of malignancies or deaths found in the corresponding strata in the general population. **Results:** With a median follow-up of 3.7 (range 0-9.9) years, 65 (7.5%) patients developed 75 second cancers (non-hematologic), 49 of these of invasive type. Compared to expected rates in the background population matched by age, sex, region of residence (6 regions) and calendar year, the risk of second malignancies was significantly higher in the CML cohort, with a Standardized Incidence Ratio (SIR) of 1.5 (95 % CI 1.13-1.99). SIR before and after the second year following diagnosis of CML was 1.6 (95 % CI 1.004-2.38) and 1.5 (95 % CI 0.98-2.11), respectively. Looking at CML subpopulations, the increased risk of developing a second malignancy reached statistical significance for females (SIR: 1.8; 95 % CI 1.18-1.99), but not for males (SIR: 1.3; 95 % CI 0.85-1.91), and for patients above 60 years of age at diagnosis (SIR: 1.5; 95 % CI 1.05–1.96). Assessment of risk by cancer type was hampered by small numbers. However, the data at hand indicate an increased risk for gastrointestinal cancer (SIR: 3.0; 95 % CI 1.60-5.16), as well as nose and throat cancer (SIR: 37.1; 95 % CI 7.46-108.40), table 1. **Conclusions:** Utilizing large, population-based registries with data accumulated during the TKI era, our results indicate that CML patients, compared to the normal control population, are at an 50% increased risk of developing a second malignancy. Similar SIR before and after the second year following the diagnosis of CML may indicate that these findings are linked to the CML disease itself, rather than to the TKI treatment. Further studies and longer follow-up seem however warranted. Physicians caring for CML patients should be aware of signs and symptoms of other malignancies in this patient population.

Table 1. Standardized Incidence Ratios for second malignancies (excluding cases of non-melanoma skin cancer, AML, ALL and MDS) among 868 Swedish CML patients diagnosed between 2002 and 2011. Total follow up time 3293 person-years (median 3.7 years).

Variable	Observed	Expected	SIR (Observed/Expected)	95 % CI for SIR
Overall	52	34	1.5	1.13–1.99
Men	26	20	1.3	0.85–1.91
Women	26	14	1.8	1.18–2.66
Age <60 years	10	5	1.9	0.89–3.42
Age ³ 60 years	42	28	1.5	1.05–1.96
Second cancer type				
Prostate	14	8	1.8	0.96–2.94
Gastrointestinal	13	4	3.0	1.60–5.16
Gynecological	4	1	3.6	0.98–9.30

Nose and Throat	3	0,1	37.1	7.46-108.40
Lung	2	2,7	0.7	0.08-2.67
Breast	4	4,2	0.98	0.26-2.45

[155] Defining Therapy Goals for Major Molecular Remission in Chronic Myeloid Leukemia: Results of the Randomized CML-Study IV. *Saussele.* ***Background****:* In the current ELN recommendations (Baccarani et al., Blood 2013) the optimal time point to achieve major molecular remission (MMR) is defined at 12 months after diagnosis of CML. MMR is not a failure criterion at any time point leading to uncertainties when to change therapy in CML patients not reaching MMR after 12 months. ***Aims****:* We sought to evaluate a failure time point for MMR using data of the CML-Study IV, a randomized five-arm trial designed to optimize imatinib therapy alone or in combination. In addition the optimal time-point to achieve a MMR should be evaluated. ***Methods****:* Patients with valid molecular analysis on MR4 level were divided randomly into a learning (LS) and a validation sample (VS). For the LS, MR2 (defined as BCR-ABL<1% which corresponds to complete cytogenetic remission (Lauseker et al. 2014)), MMR and deep molecular remission levels (MR4 or deeper) monthly landmarks were defined between one and five years after diagnosis. A patient was considered to be in MR2, MMR or MR4 from the first diagnosis of the corresponding remission level and could only change to a higher level of response. Patients were censored after SCT. The best prediction time was found via “dynamic prediction by landmarking” (van Houwelingen, Scand J Stat 2007). For the failure time point analysis, for each of the resulting 48 landmarks, a Cox model was used to define the time to progression with age and EUTOS score as additional prognostic factors. Additionally, the regression coefficients of the model of one landmark were converted to hazard ratios (HR) and treated as dependent on the HRs of the other landmarks, using a cubic smoothing function (see Fig 1). The minimum of this function was considered to be the optimal landmark point for the prediction of progression-free survival (PFS). For the calculated time point, landmark analysis for probability of PFS (defined as appearance of accelerated phase, blast crisis or death) was performed in the VS. For the evaluation of the optimal time point of achieving a MMR the same analysis was done from 0.25 to 5 years to define the time to MR4 or deeper. ***Results****:* 1551 patients were randomized from 2002 to 2012, 1358 had a valid molecular analysis on the MR4 level. 114 patients in the imatinib after IFN arm and 16 patients with missing EUTOS score were excluded. Of the 1228 evaluable patients two thirds were randomly allocated to the LS (n=818) and one third to the VS (n=410). Percentage of patients of the LS in MR2, MMR and MR4 or deeper at one year was 28%, 29% and 14%, and at 5 years 5%, 21% and 71%, respectively. Monthly time points in between were also calculated. 44 patients of the LS reached MMR on second generation tyrosine kinase inhibitors.. The minimum of the cubic function of the HRs was found for MMR at 2.34 years with a HR of 0.25 (compared to patients without any remission) and 0.75 compared to those in MR2. For MR4 or deeper no exact time point could be calculated (see Fig. 1), although it was shown that the risk of progression was slightly lower for MR4 than for MMR. Since the time interval for molecular evaluation in the study is 3 months, the validation was done with 2.25 instead of 2.34 years. 364 of the 410 of the VS were still at risk at this time point and evaluable. A significant PFS advantage for patients in MMR could be demonstrated (p=0.018). At 8 years, the probability of PFS for patients in MMR was 90.8% (confidence interval 87.0-93.7%) vs. 80.5% (confidence interval 70.2-88.6%) for patients not in MMR (see Fig 2). For the optimal MMR analysis no singular time point could be calculated as the earlier a MMR was reached the higher was the chance to achieve a MR4. ***Conclusions****:* In this model, an optimal time point to predict PFS in patients with MMR was defined at 2.25 years after diagnosis and could be validated as significant. Nevertheless, patients being in MMR had a lower risk of progression than patients not being in MMR on any other time point as well. With this model we can give hints when to define MMR as failure and a change in therapy should be considered. Despite this we should keep in mind that the earlier MMR was achieved the higher was the chance to achieve deep molecular response later during therapy.

[156] Comparing the Prognostic Significance of Early Predictors of Survival in Chronic Myeloid Leukemia (CML) Treated with Imatinib - an Analysis of the Randomized CML-Study IV. *Hanfstein.* **Introduction:** Early prediction of outcome using response-related predictive landmarks has become a major paradigm in the clinical management of chronic myeloid leukemia (CML). Several studies have shown the predictive impact of 10% BCR-ABLIS at 3 and 6 months for different tyrosine kinase inhibitors. The question, which landmark should define treatment failure and determine treatment intervention has been discussed vividly. However, an objective analysis of quality criteria for different early prognostic landmarks is lacking up to now. Here we compare sensitivity, specificity and the proportion of later disease progressions predicted by 3-month and 6-month landmarks in imatinib-treated patients of the CML-study IV. **Methods:** A total of 1,303 newly diagnosed patients were assigned to an imatinib-based treatment arm of CML-Study IV by April 2010. Median follow-up was 7.1 years. The number of molecular assessments was as follows: n=789 (at 6 months), n=692 (at 3 months) and n=301 (at 3 months and at diagnosis, without pretreatment). Gene expression levels were determined by quantitative RT-PCR. At 3 and 6 months, a BCR-ABL ratio was calculated using ABL as reference gene and standardized according to the international scale (BCR-ABLIS). In addition, at 3 months and at diagnosis a BCR-ABL ratio was calculated using beta-glucuronidase (GUS) as reference gene in order to ensure linearity of measurement at diagnosis. The log reduction at 3 months was calculated from the BCR-

ABL ratio at 3 months and at diagnosis. Due to the time-dependent nature of censored survival data, the sensitivity and specificity at eight years were calculated using the method by Heagerty et al. (Biometrics 2000). Overall survival (OS) is defined by the absence of death from any reason, progression-free survival (PFS) is defined as survival in the absence of progression to accelerated or blastic phase. Landmark analyses were performed to compare survival outcomes according to Kaplan-Meier. **Results:** Comparing the 10% BCR-ABLIS landmark at 3 and 6 months, 8-year OS and PFS rates are equal or comparable (table). In contrast, sensitivity and specificity differ substantially with an advantage in favor of sensitivity for the 3-month landmark and in favor of specificity for the 6-month landmark. This difference is paralleled by a smaller proportion of high-risk patients and less progressions identified by the 6-month landmark. From a clinical point of view the 6-month landmark is not only less than half as sensitive, moreover a treatment intervention at 6 months might also prevent less progressions due to the delay of 3 months. The half-log reduction landmark at 3 months is as sensitive as 10% BCR-ABLIS at the same time. However, it shows improved specificity and defines the smallest proportion of high-risk patients. **Conclusion:** The 10% BCR-ABLIS landmark, which is currently defining treatment failure at 6 months according to European LeukemiaNet (ELN) criteria, fails to detect the majority of patients with later disease progression. Less than a half-log reduction of individual baseline BCR-ABL transcript levels at 3 months on treatment identifies patients with later progressions as sensitive but with higher specificity as compared to 10% BCR-ABLIS.

3 Strategies to Circumvent Therapy Resistance [397-402]

[397] Induced Pluripotent Stem Cell Model of Chronic Myeloid Leukemia Revealed Olfactomedin 4 As a Novel Survival Factor for Primitive Leukemia Cells. *Kran Suknuntha*. CML is a myeloproliferative disorder characterized by unregulated growth of predominantly myeloid cells, and their subsequent accumulation in the bone marrow and peripheral blood. CML originates in hematopoietic stem cells (HSCs) with t(9;22)(q34;q11.2) translocation, which causes the constitutively expression of the BCR-ABL kinase driving the expansion of leukemic progeny. *Ex vivo* cultures of CML-derived cell lines and primary CML cells, ectopic expression of *BCR-ABL* in CD34+ cells and mouse models have provided important insights into CML pathogenesis and led to the development of targeted therapy for this neoplastic disease with BCR-ABL thyrosine kinase inhibitor (TKI), imatinib. Despite these achievements, in many cases CML remains incurable because of innate resistance of CML leukemia stem cells (LSCs) to TKI. Thus, a definitive cure for leukemia requires identifying novel therapeutic targets to eradicate LSCs. However, the rarity of LSCs within the pool of malignant cells remains a major limiting factor for their study in humans. Recently we generated transgene-free iPSCs from the bone marrow mononuclear cells of a patient in the chronic phase of CML (CML15 iPSCs and CML17 iPSCs) and showed that these iPSCs capture the entire genome of neoplastic cells, including the unique 4-way translocation between chromosomes 1, 9, 22, and 11 that was present in the patient bone marrow (BM) (Hu et al., Blood 2011). By differentiating CML iPSCs back to the blood we were able to generate iCD34+ primitive hematopoietic cells with typical LSC properties, including HSC phenotype (lin-CD34+CD45+CD90+CD117+CD45RA-RholowALDHhigh), adhesion defect, increased long-term survival and proliferation, and innate resistance to TKI imatinib. By analyzing transcriptome of CML and normal BM iCD34+ cells treated or non-treated with imatinib we discovered OLFM4 as top-ranking gene, which is selectively upregulated by imatinib in CML, but not normal BM iCD34+ cells. Using siRNA, we demonstrated that OLFM4 knockdown potentiate imatinib-induced apoptosis and suppression of CFCs in iCD34+ cells, thereby indicating that OLFM4 is involved in regulation of imatinib resistance and survival of *de novo* generated primitive CML cells. To find out whether findings obtained using iCD34+ cells can be translated to somatic cells, we evaluated the expression and functional role of OLFM4 in CD34+ cells obtained from parental bone marrow and bone marrow from the several other CML patients in the chronic phase. Using immunohistochemistry and RT-PCR we confirmed OLFM4 expression in lin-CD34+ and CD34- bone marrow cells from patients. Knockdown OLFM4 with siRNA in somatic CML lin-CD34+ potentiated imatininb-induced CFC suppression, abrogated LTC-ICs and engraftment of lin-CD34+ cells in NSGW41 mice, thereby indicating that OLFM4 is critical for survival of CML LSCs. In summary, we showed that reprogramming leukemia cells to pluripotency and then differentiating them back into blood cells can be used as a novel approach to produce an unlimited number of primitive hematopoietic cells with LSC properties and identify of novel LSC survival factors and drug targets. We validated this approach by demonstrating the successful application of the iPSC-based platform to discover OLFM4 as a novel LSC survival factor in patients in the chronic phase of CML.

[398] ABL001, a Potent Allosteric Inhibitor of BCR-ABL, Prevents Emergence of Resistant Disease When Administered in Combination with Nilotinib in an *in Vivo* Murine Model of Chronic Myeloid Leukemia. *Andrew Wylie.* ***Background***: Chronic myelogenous leukemia (CML) and a subset of acute lymphoblastic leukemia (ALL) are caused by the t(9;22)(q34;q11.2) chromosome translocation, resulting in fusion of the BCR and ABL1 genes on the Philadelphia chromosome to encode constitutively active ABL1 kinase. Despite the dramatic progress made over the past decade with tyrosine kinase inhibitors (TKIs) in the

treatment of CML, allogeneic stem cell transplant is considered the only proven curative therapy. To achieve cure or benefit from treatment-free remissions with pharmacologically-based therapies, it is estimated that patients will likely need to achieve a sustained reduction in tumor burden of 4 logs (MR4) or deeper (MR4.5). Currently, only 39% and 18% of patients achieve MR4 by 24 months of treatment with single agent nilotinib or imatinib, respectively. Furthermore, for a subset of CML patients and the majority of Ph+ ALL patients, resistance develops to current TKI's as a result of emergence of point mutations in the ATP site of the kinase domain. ABL001 is a potent, selective BCR-ABL inhibitor that maintains activity across most mutations, including T315I, with a distinct, allosteric mechanism of action which recently entered Phase I development for the treatment of patients with CML and Ph+ ALL. ABL001 was developed to be dosed in combination with nilotinib to provide greater pharmacological coverage of BCR-ABL disease and prevent the emergence of resistance. ***Methods****:* Based on X-ray crystallography, NMR and molecular modeling, ABL001 is the result of a structure-guided medicinal chemistry program targeting the myristoyl pocket of the ABL1 kinase. *In vitro* cell based assays were performed using the Ba/F3 isogenic cell system and a panel of over 300 cell lines. KCL-22 cells were used to develop an *in vivo* xenograft model to assess the efficacy of ABL001 and the PD marker, pSTAT5, was used to monitor the inhibition of BCR-ABL signaling. ***Results****:* In contrast to TKIs that bind to the ATP-site of the ABL1 kinase domain, NMR and X-Ray crystallography studies confirmed that ABL001 binds to a pocket on the BCR-ABL kinase domain that is normally occupied by the myristoylated N-terminus of ABL1. Upon fusion with BCR, this myristoylated N-terminus that serves to autoregulate ABL1 activity is lost. ABL001 functionally mimics the role of the myristoylated N-terminus by occupying its vacant binding site and restores the negative regulation of the kinase activity. Cell proliferation studies demonstrate that ABL001 selectively inhibited the growth of CML and Ph+ ALL cells with potencies ranging from 1-10nM range. In contrast, BCR-ABL-negative cell lines remained unaffected at concentrations 1000-fold higher. With resistance emerging in the clinic to current TKI's as a result of point mutations in the ATP-site, ABL001 was tested for activity against clinically observed mutations and found to be active in the low nM range. In the KCL-22 mouse xenograft model, ABL001 displayed potent anti-tumor activity with complete tumor regression observed and a clear dose-dependent correlation with pSTAT5 inhibition. The KCL-22 xenograft model was also used to compare the dosing of ABL001 and nilotinib as single agents to dosing a combination of ABL001 and nilotinib. Single agent dosing regimens led to tumor regressions; however, despite continuous dosing, all tumors relapsed within 30-60 days with evidence of point mutations in the resistant tumors. In contrast, animals treated with the combination of ABL001 and nilotinib achieved sustained tumor regression with no evidence of disease relapse either during the 70 days of treatment or for > 150 days after treatment stopped. ***Conclusion***: ABL001 selectively inhibited the proliferation of cells expressing the BCR-ABL fusion gene and was active against clinically important mutations that arise with current TKI therapy in CML. In an *in vivo* model of CML, the combination of ABL001 and nilotinib resulted in complete and sustained tumor regression with no evidence of disease relapse. These results provide proof-of-principle that simultaneous targeting of the myristoyl pocket and ATP-pocket by ABL001 and nilotinib, respectively, promotes a more sustained overall efficacy and prevents the emergence of resistance via acquisition of point mutations in the respective binding sites. ABL001 is currently being evaluated in a Phase 1 study in patients with CML and Ph+ ALL.

[399] Detection of *BCR-ABL1* Compound and Polyclonal Mutants in Chronic Myeloid Leukemia Patients Using a Novel Next Generation Sequencing Approach That Minimises PCR and Sequencing Errors. *Parker**. Background**. BCR-ABL1* kinase domain (KD) mutations are the most common known cause of resistance to tyrosine kinase inhibitors (TKIs) in CML. Mutation analysis is critical for selection of subsequent TKI therapy after treatment failure. Low level and compound mutants (>1 KD mutation in the same molecule) may also lead to therapy failure. However, compound and multiple polyclonal mutants cannot be distinguished by conventional methods as they determine the average genotype of all molecules. Next generation sequencing (NGS) has the potential to sensitively detect these mutants, however sequencing and PCR errors confound the detection of true, low level mutants using current approaches. Indeed, we demonstrated that the reported frequency of *BCR-ABL1* compound mutants may be over estimated due to PCR recombination artifacts that mimic compound mutations (Parker Blood 2014). More reliable methods are needed to appropriately assess the impact of various mutations on patient (pt) outcome. ***Aim.***To develop a clinically applicable NGS assay that can robustly distinguish *BCR-ABL1* compound and polyclonal mutants. ***Method.*** We have developed a novel NGS assay termed Single Molecule Consensus Sequencing (SMCS) that involves tagging individual *BCR-ABL1* cDNA molecules before library amplification, enabling identification and elimination of most PCR and sequencing errors. NGS was performed on the Illumina MiSeq, 2 x 300 bp; aa 244 - 407 of the KD was examined. Reads derived from an initial *BCR-ABL1* molecule are identified bioinformatically by virtue of sharing the same tag sequence. The consensus sequence of reads with the same tag is determined using automated variant calling and filtering algorithms. The consensus sequence represents the sequence of the initial *BCR-ABL1* cDNA molecule (Fig A). ***Results*** To test the validity of SMCS, we examined 10 samples lacking KD mutations and 5 mock samples created by mixing compound mutant plasmids or pt samples. Examination of raw sequencing reads revealed a complex spectrum of mutants, similar to previous clinical reports. SMCS enabled bioinformatic filtering of these artifacts, largely eliminating PCR and sequencing error, and exclusively reported the compound and polyclonal mutants known

to be present in the mock samples. We estimated the background error rate to be ~2x10-5 per base. The error spectrum was consistent with DNA damage causing first round PCR errors. SMCS was used to retrospectively examine samples of 46 pts (36 CP, 2 AP, 8 BP) who were resistant to ≤4 TKIs (1st and 2nd generation). 71 mutations were previously detected by Sanger sequencing in these samples, collected before starting next line TKI. Within the region examined using SMCS, there was 100% detection concordance with Sanger sequencing. We compared the results of SMCS with an amplicon NGS method performed at another centre for 24/46 pts (Ion Torrent, depth ~10000). Ion Torrent detected 34 compound mutants in 24 pts. Of the 30/34 that were within the region examined by SMCS we only detected 8. Based on observations in Parker Blood 2014, 14 of the 22 compound mutants not detected by SMCS were likely to be PCR recombination artifacts. The other 8/22 were low level (1 - 4%) and most (6/8) involved mutations rarely/never reported in TKI resistant pts so may also be artifacts (Fig B). We detected 3 additional compound mutants in these 24 pts, plus 5 in the remaining 22/46 pts. The compound mutants detected by SMCS were consistent with the pts' TKI treatment history. ***Conclusion*** We demonstrated detection of *BCR-ABL1* compound and polyclonal mutants in pt samples using a novel NGS assay that has the potential to overcome technical artifacts generated with other published methods. Whilst there is no gold standard method that can accurately detect low level compound mutations, SMCS has correctly identified sequencing and PCR recombination artifacts using mock samples. The accuracy and clinical utility of SMCS for sensitive compound and polyclonal mutant detection is currently being validated in another group of 200 imatinib resistant pts. The frequency of compound mutants detected in pts with >1 mutation by SMCS in the current analysis (35%) is approximately half of that reported previously, which suggests the published frequency may have been overestimated. Our novel assay takes an important step towards enabling a more concrete understanding of the mutation spectra in pts and their association with resistance.

[400] CD70/CD27 Signaling Mediates Resistance of Chronic Myeloid Leukemia Stem Cells to Tyrosine Kinase Inhibitors By Compensatory Activation of the Wnt Pathway. *Riether*. The introduction of BCR/ABL-specific tyrosine kinase inhibitors (TKIs) a decade ago revolutionized chronic myelogenous leukemia (CML) therapy. However, disease-initiating leukemia stem cells (LSCs) in CML are resistant to TKIs despite BCR/ABL inhibition. Therefore, CML will ultimately relapse upon drug discontinuation. We have previously shown that blocking CD70/CD27 signaling targets LSCs by inhibiting the activation of the Wnt pathway. Here, we investigated a combination therapy of TKIs and CD70/CD27 blocking monoclonal antibodies in human and murine CML. We demonstrate that TKI-mediated BCR/ABL inhibition down-regulates miR-29, leading to increased expression of specificity protein 1 (SP1), a transcription factor with binding site in the CD70 promoter. In addition, TKI treatment reduced the expression of DNA methyltransferases resulting in de-methylation of the CD70 promoter. These combined effects resulted in CD70 up-regulation on LSCs, enhanced CD70/CD27 signaling and compensatory Wnt pathway activation. Combined BCR/ABL and CD70/CD27 inhibition synergistically reduced Wnt signaling and eradicated leukemia cells in vitro. More importantly, combination therapy effectively eliminated CD34+ CML stem/progenitor cells in murine xenografts and LSCs in a murine CML model. Therefore, TKI-induced CD70 up-regulation triggers CD70/CD27 signaling leading to compensatory Wnt activation. These findings identify an important targetable TKI resistance mechanism of CML LSCs and may lead to new therapeutic strategies to directly target LSCs to overcome treatment resistance.

[401] Combination of Tyrosine Kinase Inhibitor with β-Catenin/CBP Modulator C82 Reverses TKI Resistance, Eradicates Quiescent CML Stem/Progenitors Cells, and Overcomes MSC-Associated Microenvironmental Protection. Zhou. Bcr-Abl tyrosine kinase inhibitors (TKIs) are effective in inducing remissions and improving survival in patients with CML but do not eliminate CML leukemia stem cells (LSCs). Wnt/β-catenin pathway is established to be active in CML and essential for CML LSC, while adult HSCs do not require fully active β-catenin for maintenance. Furthermore, Wnt/β-catenin signaling pathway plays a critical role in TKI resistance and stromal-mediated microenvironmental protection for CML stem and progenitor cells. We propose that combinations of β-catenin inhibitors and TKIs represent a potentially effective therapy by targeting both CML LSCs and leukemia-mediated microenvironmental protection. C82 is a novel β-catenin/CBP modulator that via binding to CBP inhibits the interaction of β-catenin and CBP and thus disrupts Wnt/β-catenin/CBP mediated cell proliferation and self-renewal signaling. CML cell lines and primary CML-BC patient samples were treated with combinations of C82 with different TKIs, including imatinib (IM), nilotinib (NIL), dasatinib (DAS), and ponatinib (PON). Both TKI-sensitive KBM5 (IC50=0.50±0.06μM, EC50=0.32±0.01μM, 48h) and TKI-resistant KBM5-STIT315I (IC50=1.44±0.06μM, EC50=0.36±0.09μM, 48h) cells were sensitive to C82. C82-TKI combinations synergistically induced apoptosis (C82-NIL, CI=0.30±0.07 and C82-DAS, CI=0.20±0.01 in KBM5; C82-NIL, CI=0.24±0.09 and C82-DAS, CI=0.36±0.05 in KBM5-STIT315I at 48h; respectively) and inhibited cell grwoth in both cell lines. KBM5, KBM5-STIT315I and K562 were co-cultured with normal human bone marrow derived-MSCs. Western blot showed that CML/hMSCs co-cultures increased β-catenin, CD44, and survivin proteins in CML cell lines. C82-TKI combinations induced similar degrees of cell death and proliferation inhibition with or without hMSC co-cultures, indicating the combination strategy can overcome MSC-mediated microenvironmental chemoprotection in CML. Western

blot analysis showed that C82 significantly inhibited CD44 and survivin expression which was further reduced by C82-TKI combinations in KBM5 and KBM5-STIT315I cells. C82-TKI combinations were evaluated in CML sample (n=6) from heavily-treated and TKI-resistant CML-BC patients. Four out of 6 sample harbored BCR-ABL kinase mutations, including T315I, E255K/V, and H396R. Mononuclear cells from the patients were stained with cell division tracking dye CFSE and then co-cultured with hMSCs. Flow cytometry was performed to identify CD34+CFSEbright and CD34+CFSEdim cells, as quiescent and proliferating population, respectively. When CML cells were treated without hMSC co-culture, C82-TKI combinations exerted stronger synergistic effects in CFSEbright quiescent cells (CI=0.21±0.06, 0.29±0.07, 0.48±0.15, or 0.26±0.03 for combination of C-82 with IM, NIL, DAS, or PON) compared with CFSEdim proliferating cells (CI=0.43±0.05, 0.43±0.17, 0.50±0.20, or 0.44±0.06 for combination of C-82 with IM, NIL, DAS, or PON; respectively). While under co-culture conditions, similar levels of synergy was observed in proliferating (CI=0.39±0.02, 0.23±0.02, 0.32±0.05, or 0.27±0.01 for combination of C-82 with IM, NIL, DAS, or PON) and quiescent cells (CI=0.23±0.02, 0.20±0.01, 0.39±0.10, or 0.20±0.04 for combination of C-82 with IM, NIL, DAS, or PON; respectively). C82-TKI combinations also synergistically induced cell death in CD34+38- CML cells (n=4) and yielded minimum effect on normal bone marrows CD34+ cells (n=3). *In vivo* studies are ongoing with immunodeficient NOD/SCID/IL2rγnull mice injected with CML-BC patient samples. An open-label, dose-escalation phase I/II study of PRI-724 (active metabolite of C82) for advanced myeloid malignancies (NCT01606579), including CML patients in combination with dasatinb, is enrolling patients at MD Anderson Cancer Center and other centers. Our data demonstrate that β-catenin/CBP signaling pathway plays a critical role in quiescent CML stem/progenitor cells and disruption of the β-catenin/CBP interaction with C82 could overcome MSC-mediated microenvironmental protection for not only proliferating but also quiescent stem/progenitor cells in CML. Combinations of β-catenin/CBP signaling pathway modulator C82 with TKIs represent a potentially promising strategy to tackle TKI resistance and eradicate CML stem/progenitors cells and should be further investigated in larger studies.

[402] Integrin-Linked Kinase As a Key Mediator of Stromal Cell-Enhanced Resistance of Primitive CML Cells to Tyrosine Kinase Inhibitors. *Rothe.* The human bone marrow (BM) compartment consists of a heterogeneous, multi-functional network of cells and extracellular matrix that interact with hematopoietic stem cells. Growing evidence suggests that this microenvironment also likely adds to the resistance of chronic myeloid leukemia (CML) stem cells to tyrosine kinase inhibitor (TKI) therapy *in vivo*. Integrin-linked kinase (ILK) is a serine/threonine kinase that is an important constituent of focal adhesions, a regulator of spindle formation during mitosis, and a key mediator of multiple signaling pathways. However, its potential role in the regulation of primitive CML cells and their response to TKIs is unknown. Our RNA-seq analysis revealed that ILK expression and its downstream targets, such as AKT, are highly upregulated in pre-treatment CD34+ CML stem/progenitor cells (n=6) compared to normal BM controls (n=3, $p<0.05$). We confirmed this finding by qRT-PCR analysis of CD34+ cells obtained from 28 CML patients (including some who subsequently became clinically resistant to imatinib mesylate (IM) treatment) compared to CD34+ BM cells from 9 normal adults ($p<0.05$). Elevated expression of ILK protein in CD34+ CML cells was also demonstrated by Western blot analysis (threefold, n=4, $p<0.05$). Interestingly, we also found ILK transcript levels to be much higher in the more primitive and rarer CD38- CML stem cell-enriched subset of the lin-CD34+ population as compared to the more mature and prevalent CD38+ subset or the terminally differentiated lin+CD34- cells ($p<0.01$). Both ILK transcripts and intracellular protein levels were elevated in CD34+ CML cells, but not in CD34- differentiated cells, in response to IM treatment *in vitro*, especially in the presence of expanded BM stromal cells, suggesting differential regulation of ILK expression in primitive CML cells and their more mature counterparts. We next asked whether ILK influences primitive CML cell responses to TKIs by using a clinically validated and selective ILK inhibitor (QLT0267) to suppress ILK activity in colony assays of CD34+ CML cells. QLT0267 plus TKIs significantly reduced the yield of colonies obtained compared to any single agent or combination of TKIs and this enhanced cell killing was most pronounced on cells from IM non-responders (n=6; $p<0.01$). Analysis of the lineages affected showed that the combination of IM or dasatinib (DA) with QLT0267 had a more pronounced effect on myeloid colony formation (CFU-GM), at concentrations where either or both TKIs had little effect (i.e. 85-90% inhibition vs. 30-45%). Moreover, the simultaneous suppression of ILK and BCR-ABL activities also effectively inhibited the growth of more primitive CML cells (from IM non-responder patients) when these were co-cultured with stromal cells in 6-week long-term cultures (LTC), in contrast to the lack of these effects in the presence of single TKIs or TKI combinations. Mechanistically, we found that the combination of QLT0267 with a TKI enhanced the induction of apoptosis of CD34+ CML cells in suspension cultures within the first three days (from 10-15% to 30-45% apoptotic cells relative to untreated controls) and CFSE (carboxy-fluorescein diacetate succinimidyl diester) tracking of cell division showed that QLT0267 specifically targets quiescent CML stem cells from IM-resistant patients (n=4; $p<0.05$). In addition, treatment with the ILK inhibitor alone or in combination with a TKI enhanced apoptosis of primitive CML cells *in vitro* by abolishing the protective effect of BM stromal cells observed under TKI treatment alone (25-30% apoptotic cells vs. 3-8%) and combination treatments confirmed strong synergy between DA and QLT0267 (CI value <0.3). Importantly, QLT0267 (up to 10 μM) was not toxic to normal CD34+BM cells in either short- or long-term culture systems (with and without stromal cells). Together, these

findings indicate that ILK is a critical player in the regulation of TKI response/resistance of primitive CML cells. The enhanced and selective effect obtained by dual inhibition of both targets, particularly in the presence of protective stromal cells to mimic their response within the BM microenvironment, may offer an important new therapeutic possibility.

4 Disease Progression and the Microenvironment: Therapeutic Implications [511-516]

[511] Malignant Reprogramming of Progenitors into Leukemia Stem Cells Is Enhanced By Upregulation of CD44 Transcript Variant 3 in Malignant Microenvironments. *Holm.* ***Introduction****.* Malignant reprogramming, first described in chronic myeloid leukemia (CML), occurs upon activation of the Wnt/b-catenin pathway in granulocyte-macrophage progenitors (GMPs) that gain the capacity to self-renew and contribute to the emergence of BCR-ABL1 tyrosine kinase inhibitor (TKI) resistant blast crisis CML. Deregulation of the Wnt/b-catenin target gene, CD44, plays a vital role in leukemia stem cell (LSC) maintenance in the malignant microenvironment in mouse models of CML. However, extensive alternative mRNA splicing in humans results in expression of multiple CD44 isoforms, some of which have been implicated in cancer invasion and metastasis. In this study we investigated the role of CD44 splice variant expression on human blast crisis LSC maintenance in the malignant niche. ***Methods and Results.*** *CD44 Isoform Expression Analysis* To investigate the splice isoform expression pattern of CD44, whole transcriptome RNA sequencing (RNA Seq; Illumina HiSeq 2000) was performed on FACS sorted chronic phase (CP; n=8) and blast crisis (BC; n=8) CML progenitors (CD34+CD38+Lin-) as well as their normal counterparts from cord blood (CB) (n=7) and adult peripheral blood (NPB; n=4). While whole gene expression analysis revealed upregulation of CD44 in blast crisis compared with chronic phase and normal progenitors, a plethora of CD44 transcript variants were also detected including variants 3, 4 (CD44s), 5, 6, 7, 8. Notably, RNA Seq isoform analysis detected a higher expression of CD44 transcript variant 3 in BC compared to CP and CB and NPB. Moreover, CD44 transcript variant 3 gene expression was highly expressed in undifferentied human embryonic stem cells (hESCs) while differentiated hESCs (embryoid bodies) had low expression, suggesting CD44 transcript variant 3 to be important for pluripotency. *Lentiviral CD44 Variant 3 Overexpression*. To directly determine the impact of CD44 variant 3 expression on malignant reprogramming of CP progenitors into self-renewing LSC, we developed a lentiviral human CD44 variant 3 overexpression vector and transduced CP CML progenitors. Transduced CP progenitors harbored increased expression of migration specific markers, such as osteopontin and ICAM1, as well as an upregulation of the pro-survival long isoforms of BCL2 family members BCLX and MCL1, thereby enhancing survival and replating in hematopoietic progenitor assays. Moreover, hESCs transduced with CD44 transcript variant 3 showed upregulation of pro-survival BCL2 isoforms, enhanced proliferation and as well as maintenance of an undifferentiated state, suggesting that CD44 transcript variant 3 promotes pluripotency. *Targeted Inhibition of CD44 variant 3 Expressing LSC*. Humanized RAG2-/-gc-/-mice engrafted with CD34+ BC CML patient samples showed a significant reduction of human progenitor cells post treatment with a clinical grade CD44 mAb, both alone and in combination with Dasatinib in all hematopoietic niches. Bone marrow and spleen samples from primary transplanted mice show a reduced gene expression level of CD44 and CD44 transcript variant 3 upon combination treatment of CD44 and Dasatinib. Most importantly, serial transplantation of progenitors treated with the CD44 mAb as well as in combination with Dasatinib revealed a significant reduction in LSC self-renewal capacity commensurate with a reduction in CD44 variant 3 expression. ***Conclusions***. Upregulation of an embryonic splice variant of CD44, variant 3, expands pluripotent stem cell populations and promotes malignant reprogramming of CML progenitors into self-renewing LSC. Treatment with a humanized CD44 specific mAb sensitizes CML LSC residing in malignant niches to Dasatinib. From these results CD44 mAb appears to be an excellent antibody for future combination clinical studies aimed at eradicating therapy resistant blast crisis LSC in CML. In addition, these observations strongly suggest that CD44 transcript variant 3 upregulation serves as a biomarker of progression from CP to BC as well as the generation of TKI resistant LSCs, with the potential of being a more specific target for future combination therapies.

[512] Cooperative Targeting of Bcl-2 Family Proteins By ABT-199 (GDC-0199) and Tyrosine Kinase Inhibitors to Eradicate Blast Crisis CML and CML Stem/Progenitor Cells. *Carter.* Bcr-Abl tyrosine kinase supports CML cell survival in part by regulating antiapoptotic Bcl-2 proteins such as Bcl-xL and Mcl-1. Tyrosine kinase inhibition, the front-line therapy for patients with chronic phase CML, is less effective in blast crisis (BC) patients and inactive against quiescent CML stem/progenitor cells. We reported that ABT-737, a dual Bcl-2/Bcl-xL inhibitor, induces apoptosis in BC CML cells including CD34+quiescent CML cells. ABT-199, a potent Bcl-2 specific inhibitor, has entered clinical trials for various hematological malignancies. We hypothesized that cooperative targeting of antiapoptotic Bcl-2 proteins using a combination of ABT-199 and tyrosine kinase inhibitors (TKIs) would exert enhanced activity against BC CML and CML stem/progenitor cells. Cells from patients (n=4) with TKI-resistant BC CML were treated with ABT-199, TKIs, and combinations. Although exerting low activity by itself, ABT-199 in combination with TKIs synergistically

induced apoptosis (CI<0.1) in bulk and CD34+38- cells from these patients regardless of their previous clinical responses to TKIs. The combinations had minimal activity against normal CD34+cells (n=3). Mechanistic studies demonstrated that nilotinib inhibited the expression of Bcl-xL and Mcl-1 mRNA and protein, even in cells from TKI (including nilotinib) resistant patients. Individual inhibition of Bcl-xL or Mcl-1, and even more so inhibition of both, by siRNAs increased the sensitivity of cells to ABT-199, suggesting that cooperative inhibition of Bcl-2 by ABT-199 and Bcl-xL/Mcl-1 by TKIs contributes to the synergy. To evaluate the effect of these combinations on TKI-insensitive quiescent stem/progenitor CML cells, BC CML patient cells were stained with the cell division-tracking dye carboxyfluorescein succinimidyl ester (CFSE) and then co-cultured with human bone marrow (BM)-derived mesenchymal stromal cells (MSCs). Once proliferating and quiescent cells were distinguishable by flow cytometry, cells were treated with ABT-199, TKIs, and their combinations for 48 hours with or without MSC co-culture. Apoptosis was measured in proliferating and quiescent progenitor cells, defined as the percentage of annexin V positivity in CD34+CFSEdim and CD34+CFSEbright cells, respectively. ABT-199 as a single agent decreased viability of CML cells cultured alone or co-cultured with MSCs in both proliferating (IC50=191±103nM and 194±64nM, respectively) and quiescent (IC50=221±75nM and 205±123nM, respectively) CD34+ CML cells. Combinations of ABT-199 with TKIs, including imatinib, nilotinib, dasatinib, or ponatinib, synergistically induced death (CI<0.2) and decreased the number of viable cells in proliferating as well as quiescent CD34+progenitor cell populations (n=6). All 6 patients were resistant to TKIs, and 4 had mutations in the BCR-ABL gene, including three with the T315I mutation. To further test the ability of ABT-199 and TKI combinations to eradicate CML stem cells, we used an inducible transgenic CML mouse model in which the BCR-ABL gene is expressed under control of a tet-regulated enhancer of the murine stem cell leukemia (Scl) gene, allowing targeted BCR-ABL expression in stem/progenitor cells. Once BM cells from transgenic Scl-tTa-BCR-ABL/GFP mice were engrafted in wild type recipient mice, the mice were treated with ABT-199, nilotinib, or both. At the end of a 3-week treatment period, each single agent alone, and even more so with the combinations, significantly decreased blood total GFP+ WBC (12.9±1.4, 5.2±0.3, 6.1±0.4, and 1.6±0.3 x106/ml in controls, ABT-199, nilotinib, and combination, respectively) and neutrophils (1.43±0.03, 0.49±0.06, 0.32±0.03, and 0.25±0.05 x106/ml in the respective groups). ABT-199 (*P*=0.02), and more so with the combination (*P*<0.01) but not nilotinib alone (*P*=0.29), significantly decreased BM GFP+ LSK cells (12.0±1.2, 6.8±0.6, 9.5±1.6, and 2.2±0.2 x103 cells in the respective groups). The *in vivo* experiments are ongoing. **Conclusions**: ABT-199 and TKIs cooperatively target antiapoptotic Bcl-2 family proteins. This combination is highly effective in killing bulk and CD34+38- CML cells and quiescent CD34+ CML stem/progenitor cells from BC CML patients *in vitro* and in suppressing leukemia and leukemia stem cells *in vivo*. This strategy has the potential to eradicate BC CML cells and CML stem/progenitor cells, neither of which are effectively targeted by TKIs alone.

[513] *BACH2* promotes Lineage-Specific Fate Decisions in *BCR-ABL1*-Driven Leukemia. *Park*. **Background and Hypothesis:** CML and Ph+ ALL are both driven by the oncogenic *BCR-ABL1* tyrosine kinase and CML can progress into lymphoid blast crisis (LBC), which is clinically and biologically indistinguishable from *Ph*+ ALL. We hypothesize that the stark differences in phenotype and clinical outcomes between CML and *Ph*+ ALL/LBC are due to lineage-specific transcription factors. Our group recently identified *BACH2* as a B cell-specific transcription factor that mediates negative selection at the pre-B cell receptor checkpoint and functions as a tumor suppressor in *Ph*+ ALL and LBC (Swaminathan et al., *Nature Med* 2013). Here we report the surprising finding that *BACH2* mediates lineage-specific fate decisions in BCR-ABL1 driven leukemia: while a potent tumor suppressor in B cell lineage in *Ph*+ ALL and LBC, *BACH2* is required for survival and self-renewal of myeloid lineage CML cells. **Results**: A gene expression analysis in 99 patients with CML (Radich et al, 2006) revealed that patients with higher blast counts showed significantly higher gene expression values of *BACH2* (*P*=8.88 x 10-9), suggesting that BACH2 mRNA levels increase with CML progression. To determine the mechanistic role of *BACH2* in the progression of CML, we studied genetic deletion of BACH2 in a CML mouse model. To this end, we transformed Lin-kit+Sca-1+(LSK) cells from *Bach2*+/+ and *Bach2*-/- bone marrow with *BCR-ABL1*. We compared these cells alongside with *Bach2*+/+ and *Bach2*-/- pre-B cells that were transformed with *BCR-ABL1* as a model for *Ph*+ ALL and LBC. *Bach2*+/+ and *Bach2*-/- CML-like and *Ph*+ ALL/LBC-like leukemia cells were then tested in a series of functional experiments to test their ability to initiate fatal leukemia in transplant recipients. While deletion of BACH2 accelerated B cell lineage leukemia (*Ph*+ ALL/LBC-like) in transplant recipients, we noted the opposite outcome in transplant experiments with myeloid lineage CML cells: All mice receiving *Bach2*+/+ *BCR-ABL1*-transformed LSK cells developed CML-like disease within 60 days whereas recipients of *Bach2*-/- cells did not develop CML-like disease (*Bach2*+/+ vs *Bach2*-/-, median survival time= 33 days vs undefined; *P*=0.001). Additionally, we observed that deletion of *Bach2* loss reduced both the colony formation ability (*P*=0.0059) and the S phase proliferation potential (*P*=0.0075) of the CML progenitors. These results were in contrast to those observed in *Ph*+ *Bach2*+/+ and *Bach2*-/- ALLs. Reconstitution of Bach2 in Bach2-/- CML cells rescued its colony forming ability (*P*= 0.023). These findings were confirmed in primary human CML cells from two patients with CML in chronic phase: Inducible overexpression of Bach2 in patient-derived CML cells conferred a selective proliferative advantage of BACH2 overexpressing cells over time, in comparison to empty vector controls. Bach2 triggered a survival program in human and mouse CML cells, in

contrast to B cell lineage *Ph*+ ALL and LBC cells. Interestingly, Bach2 did not provide a selective growth advantage to untransformed myeloid and multi-lineage progenitor cells. This demonstrates that Bach2 drives lineage-specific fate decisions and cooperates with the transforming BCR-ABL1 oncogene. Utilizing an *in vitro* lineage-switch assay overexpressing CEBPα we characterized the opposing roles of BACH2 in *Ph*+ ALL and CML. Cell viability and colony formation assays demonstrated that absence of Bach2 results in increased survival and proliferation of *Ph*+ ALL cells. In striking contrast, however, CEBPα-induced myeloid reprogramming induced cell cycle arrest and suppressed colony formation in Bach2-deficient cells (*P*=0.0001). **Conclusion**: While BACH2 is primarily expressed in the B cell lineage, functioning as a tumor suppressor in *Ph*+ ALL, we report here the surprising finding that *BACH2* is required for survival and self-renewal of myeloid lineage CML cells. *BACH2* mediates lineage-specific fate decisions in BCR-ABL1 driven leukemia and represents one example of how conversion of lineage identity (e.g. from CML to LBC) may impact specific requirement for malignant transformation. For instance, deletions of BACH2 at 6q15 are common in CML at the time of LBC progression but are not found in CML chronic phase or myeloid blast crisis.

[514] Pyrvinium Selectively Targets Blast Phase Chronic Myeloid Leukaemia through Inhibition of Mitochondrial Respiration. *Xiang.* The use of Bcr-Abl tyrosine kinase inhibitors (TKIs) has led to excellent clinical responses in patients with chronic phase chronic myeloid leukaemia (CML). However these TKIs have been less effective as single agents in blast phase (BP) CML and this represents an urgent unmet need. A number of novel agents are now being investigated but most have not been translated to the clinics yet. Pyrvinium, a FDA-approved anthelminthic drug, was reported to selectively inhibit growth of a number of tumour cell types including myeloma and erythroleukaemia. We investigated the effect and mechanism of action of pyrvinium in BP-CML. Our results show that pyrvinium selectively targeted BP-CML CD34+ progenitor cells. It induced apoptosis in CD34+ cells from TKI-resistant BP-CML patients who harbour Bcr-Abl kinase mutations, while sparing normal cord blood CD34+ cells. In addition, pyrvinium was more effective in inhibiting colony formation and self-renewal capacity in BP-CML CD34+ cells than cord blood CD34+ cells. Further increase in apoptosis, decrease in colony formation and self-renewal were seen when dasatinib was combined with pyrvinium in BP-CML but not cord blood CD34+ cells (Figure 1 A-C). We also showed that pyrvinium was synergistic in combination with dasatinib in inhibiting proliferation and inducing apoptosis in CML cell lines. We next tested the effects of pyrvinium and its combination with dasatinib in a CML xenograft model and showed that pyrvinium significantly delayed tumour growth with no signs of toxicity in the mice. When combined with dasatinib, the tumour growth was completely inhibited (Figure 1D). These results indicate that pyrvinium is active in BP-CML *in vitro* and *in vivo*. The anti-cancer effects of pyrvinium have been reported to due to allosteric activation of casein kinase 1α (CK1α) and suppression of Wnt/β-catenin signalling (Thorne et al, Nature Chemical Biology 2010). However we showed that despite the efficient knockdown of CK1α and overexpression of β-catenin, the proliferation inhibitory effect of pyrvinium was not abolished, indicating a CK1α and β-catenin-independent mechanism of action in BP-CML. Instead, we observed that pyrvinium preferentially localized to mitochondria in CML cells and that pyrvinium inhibited oxygen consumption of CML cells within 5 minutes of treatment. In mitochondrial respiratory chain-deficient CML ρ0 cells which lack mitochondrial DNA and have undetectable oxygen consumption, we showed that the effect of pyrvinium in reducing ATP levels and inducing apoptosis was abolished. These results indicate that pyrvinium acts in CML through the inhibition of mitochondrial respiration. We have shown that pyrvinium alone and in combination with Bcr-Abl TKI selectively targets BP-CML progenitor cells and pyrvinium acts in BP-CML through the inhibition of mitochondrial respiration. Given that pyrvinium is already clinically available, our pre-clinical findings can be translated rapidly into the clinics. Our data also suggests that targeting mitochondrial respiration may be a potential therapeutic strategy in aggressive leukaemia.

[515] Evaluation of Extrinsic and Intrinsic Cues Involved in BCR-ABL-Induced Leukemogenesis. *Sontakke.* Intrinsic and extrinsic signals together contribute to determine self renewal, quiescence or the specific metabolic status of leukemic stem cells (LSC) in BCR-ABL mediated chronic myeloid leukemia (CML). Our previous studies have shown that expression of BCR-ABL together with the polycomb repression complex 1 member BMI1 in human CD34+ cells is sufficient to induce a serially transplantable lymphoid leukemia in vivo while a myeloid phenotype was never observed. Yet in vitro, both lymphoid as well as myeloid immortalized long-term cultures could readily be established, in line with phenotypes observed in CML patients. Since NSG models are typically lymphoid biased due to the absence of species-specific myeloid growth factors, we hypothesized that extrinsic factors might dictate lineage fate. Using a “humanized” NSG mouse model in which scaffolds seeded with human mesenchymal stromal cells were implanted we observed that, in contrast to the murine niche, BCR-ABL overexpression alone was sufficient to induce a serially transplantable leukemia of both the lymphoid and myeloid lineage. Using myeloid blast-crisis CML patient cells, engraftment was also observed whereby the immature blast-like phenotype was predominantly maintained in the humanized scaffold niche, and to a much lesser extent in the murine niche. This distinction could also be demonstrated functionally by using in vitro long-term self-renewing cultures. Blast cells retrieved from the human scaffold niche could readily be established while no long-term cultures could be initiated from

cells retrieved from the murine bone marrow niche. Genome-wide transcriptome analyses of leukemic cells retrieved from the mouse BM niche and from the human scaffold niche revealed striking differences in gene expression imposed on BCR-ABL+ cells by these different environments. For example, endogenous BMI1 levels were significantly higher in BCR-ABL cells retrieved from human scaffold niche as compared to murine BM harvested cells suggesting that BMI1 might still be required as additional factor to prevent oncogene-induced senescence. Apart from epigenetic modifiers, we hypothesized that the hypoxic microenvironment might play an important role in maintaining CML LSCs and studied that in detail. Hypoxia inducible factor 1α (HIF1) and HIF2 act as transcription factors that are stabilized under hypoxic conditions. HIF1 has been characterized as an important factor that controls cellular metabolism while the role of HIF2 is still less clear. Earlier we identified HIF2 as downstream target of STAT5 and observed elevated glucose uptake in STAT5 activated HSCs. Several genes associated with glucose metabolism were upregulated by STAT5 in an HIF2 dependent manner, including SLC2A1 and GYS2. Here, we investigated metabolic changes in BCR-ABL expressing human stem/progenitor cells and focused on the role on HIF1 and HIF2. Genome-wide transcriptome analyses were performed on human CB CD34+ cells transduced with BCR-ABL as well as on BCR-ABL-positive CML and B-ALL patient samples. GSEA analyses indicated that these transcriptome changes were strongly enriched for STAT5 and MYC signatures as well as for hypoxia, embryonic stem cell and glucose metabolism gene signatures which included upregulation of e.g. SLC2A3, SLC2A1 and HIF1 and HIF2. These data suggest that BCR-ABL imposes hypoxic signaling under normoxic conditions. Moreover, downregulation of HIF1 and HIF2 using a shRNA approach impaired proliferation and reduced progenitor frequencies of BCR-ABL+ cells. Next we studied metabolic changes in BCR-ABL+ cells using NMR spectroscopy. We observed striking differences in uptake and secretion of metabolites when BCR-ABL CB CD34+ cells were compared to normal CB CD34+ cells under normoxia and hypoxia. As expected, BCR-ABL cells exhibited enhanced glycolysis as determined by an increased production and secretion of lactate under both normoxic and hypoxic conditions. Interestingly, glutamine levels were strongly enhanced in BCR-ABL+ cells, in a HIF1/2-dependent manner, possibly via enhanced glutamine import or glutamine production via upregulation/activation of Glutamine Synthase. Our current hypothesis is that BCR-ABL+ cancer cells make use of enhanced glutamine metabolism to maintain TCA cell cycle activity in glycolytic cells, and studies focus on whether targeting this pathway might provide alternative means to eradicate LSCs.

[516] The Vascular Niche Is Involved in Regulating Leukemic Stem Cells in Murine Chronic Myelogenous Leukemia. *Aggoune.* Chronic myelogenous leukemia (CML) is effectively controlled by tyrosine kinase inhibitors (TKIs) such as imatinib mesylate, leading to a hematologic remission in >90% of patients. However, the majority of patients relapse once TKI therapy is discontinued, suggesting that CML leukemia stem cells (LSC) are not eradicated. We recently showed that modulation of the osteoblastic niche can lead to reduction of LSC in CML (Krause et al., Nat. Med. 2013;19:1513), but the role of the vascular hematopoietic stem cell niche in CML has not been well defined. E-selectin is expressed on bone marrow (BM) endothelium within the vascular niche, whereas loss of E-selectin expression or treatment with GMI-1271, an E-selectin small molecule antagonist, enhances HSC quiescence and self-renewal (Winkler et al., Nat. Med. 2012;18:1651). E-selectin also plays a critical role in the homing and engraftment of CML LSCs (Krause et al., Blood 2014;123:1361) through E-selectin ligands expressed on the LSCs, including CD44 (Krause et al., Nat Med. 2006;12:1175). We, therefore, hypothesized that E-selectin blockade with GMI-1271 may overcome niche-mediated resistance to TKIs and eradicate CML LSC. Using the well-described murine retroviral transduction/transplantation model of CML we showed that the white blood cell count (WBC) of mice with BCR-ABL1-induced CML-like leukemia was significantly reduced by treatment with imatinib plus GMI-1271 (or imatinib alone) and there was a trend towards WBC reduction by treatment with GMI-1271 alone (*P*=0.07). The percentage of GFP+ Mac-1+ cells in peripheral blood on day 16 post-transplant was decreased by imatinib or GMI-1271 alone or by combined treatment with imatinib and GMI-1271. Spleen weights were significantly reduced by combined treatment with imatinib plus GMI 1271. Furthermore, the BM GFP+ (*BCR-ABL1*+) Lin– c-Kit+ Sca-1+ population, which contains the LSCs in this model, was significantly reduced in animals treated with GMI-1271 compared to vehicle controls. As expected, treatment with imatinib alone had no effect on BM LSC frequency, and there was no added benefit in the reduction of LSC when imatinib and GMI-1271 were combined. In addition, the survival of mice treated with imatinib plus GMI-1271 was significantly prolonged compared to vehicle-treated animals, with ~20% of mice treated with GMI-1271 alone or the combination of imatinib and GMI-1271 exhibiting long-term low-burden disease despite discontinuation of treatment on day 28 post-transplant. In these primary recipients neither *BCR-ABL1*+ myeloid cells nor *BCR-ABL1*+ LSC were mobilized to peripheral organs. However, fewer *BCR-ABL1*+ LSC were found in the spleen of mice treated with GMI-1271 compared to imatinib-treated mice. There was a significant reduction in the frequency of cycling *BCR-ABL1*+ LSC in mice treated with GMI-1271 and imatinib. To assess directly the effect of E-selectin inhibition on LSC frequency and function, we transplanted BM from primary leukemic mice treated with vehicle, imatinib, GMI-1271 or the combination of imatinib and GMI-1271 into irradiated secondary recipient mice. There was a significant reduction of WBC and a trend towards reduction of *BCR-ABL1*+ myeloid cells in secondary recipients of BM from donors treated with GMI-1271 alone or in combination with imatinib, but not by imatinib alone. These data suggest that modulation of the

vascular niche and, specifically, inhibition of E-selectin may be a possible strategy to target LSC in CML, possibly via a reduction in S-G2/M as cells arrest prior to apoptosis, even when imatinib is discontinued. Further studies on the effects of GMI-1271 on homing of LSC and the more exact mechanism of LSC reduction by GMI-1271 are being performed.

5 Prognosis and Therapy [517-522]

[517] SPIRIT 2: An NCRI Randomised Study Comparing Dasatinib with Imatinib in Patients with Newly Diagnosed CML. *O'Brien.* **Objective**. SPIRIT 2 is the largest phase 3 prospective randomized open-label trial comparing imatinib 400mg with dasatinib 100mg daily: this is the first presentation of data comparing the two arms. **Methods**. 814 patients were recruited at 144 hospitals between August 2008 and March 2013. 812 started study medication (406 in each arm). The primary endpoint is event-free survival at 5 years. A key secondary endpoint is the rate of achievement of a BCR-ABL/ABL ratio of <0.1%IS (major molecular response (MMR), 3 log reduction or MR3). **Results**. *Discontinuations*. With a median follow up of 34 months a total of 289/812 (35.6%) patients have discontinued study medication. 118/812 (14.5%) patients have discontinued due to non-haematological toxicity: imatinib 47/406 (11.6%); dasatinib 71/406 (17.5%). 40 patients discontinued due to sub optimal response as assessed by the treating physician: imatinib 37/406 (9.1%); dasatinib 3/406 (0.7%). *Side effects.* Patients receiving imatinib experienced GI toxicity more often than patients receiving dasatinib; fatigue, rash and headache were more common with dasatinib. A higher rate of grade 3/4 thrombocytopenia was observed in the dasatinib arm: imatinib 17/406 (4.2%); dasatinib 52/406 (12.8%). Pleural effusions occurred in 78/406 (19.2%) patients on dasatinib; 13 of 78 (16.7%) patients required drainage. Arterial cardiovascular events (excluding hypertension) were experienced by 10/812 (1.2%) patients: imatinib 2/406 (0.5%; myocardial infarction (MI) x2); dasatinib 8/406 (2.0%; MI x1; angina/acute coronary syndrome x5; peripheral arterial disease x2). Hypertension was observed in 10/812 (1.2%) patients: imatinib 3/406 (0.7%); dasatinib 7/406 (1.7%). Venous CV events occurred in 7/812 (0.9%) patients: imatinib 3/406 (0.7%); dasatinib 4/406 (1.0%). *Efficacy.* For both PCR and cytogenetic analyses patients that had discontinued their allocated therapy or that did not have a 12 month sample were analysed as not having achieved MR3/CCR. The MR3 (PCR <0.1% IS) rate at 12 months in all treated patients is significantly different (p<0.001) between the two treatment arms: imatinib 173/406 (42.6%); dasatinib 236/406 (58.1%). The MR3 rate at 12 months in patients treated with dasatinib is 51/78 (65.4%) in those with a pleural effusion and 185/328 (56.4%) in those without (p=0.148, NS). The complete cytogenetic response (CCR) rate at 12 months is: imatinib 163/406 (40.1%); dasatinib 207/406 (51.0%). The difference between the two treatment arms is statistically significant (p=0.002) but caution is required in interpreting these data as there were missing analyses in 367 of 812 (45.2%) patients: imatinib 191 of 406 (47.0%), dasatinib 176 of 406 (43.3%). The difference in major cytogenetic response (MCR) rate between the two treatment arms at 12 months is not statistically significant: imatinib 200/406 (49.3%); dasatinib 218/406 (53.7%), p=0.206. *Disease progression and deaths.* 16 patients have progressed to either accelerated phase or blast crisis and 13 of those progressions were within the first year. Accelerated phase: imatinib 3/406 (0.7%); dasatinib 2/406 (0.5%). Blast crisis: imatinib 7/406 (1.7%); dasatinib 4/406 (1.0%). **Conclusions**. Dasatinib-treated patients have a higher rate of molecular response at 1 year but, with a median of 34 months follow up, there is no significant difference in rates of disease progression or overall survival. More patients abandoned imatinib than dasatinib due to investigator concerns about sub optimal responses. Further follow up is required to evaluate whether there will be differences in event free survival at five years.

[518] Achieving Early Landmark Response Is Predictive of Outcomes in Heavily Pretreated Patients with Chronic Phase Chronic Myeloid Leukemia (CP-CML) Treated with Ponatinib*. Mueller.* **Background**: Ponatinib is an approved potent, oral, pan-BCR-ABL inhibitor active against native and mutant BCR-ABL. Ponatinib had substantial clinical activity in the phase 2 PACE trial in patients (pts) resistant or intolerant to dasatinib or nilotinib or with the T315I mutation. In the frontline setting, positive associations between achieving response at an early time point (landmark) and long-term outcomes have been shown for tyrosine kinase inhibitors (TKIs) in CML pts. Since landmark analyses have not been reported for a heavily pretreated population, in particular 3rd line and beyond, this retrospective analysis investigated the impact of achieving early landmark responses with ponatinib on long-term outcomes in PACE pts. **Methods**: Ponatinib treated CP-CML pts in PACE with valid cytogenetic and molecular assessments were included. Pts who met response criteria at entry, were missing assessments or not evaluable (<20 [13] metaphases examined for CCyR [MCyR]) at time of response assessment, who dropped out or who progressed (for PFS analysis) prior to response assessment were excluded. Pts were classified by molecular status (BCR-ABLIS: ≤0.1% [MMR], ≤1%, ≤10% and >10%) and cytogenetic status (MCyR, <35% Ph+ metaphases; CCyR, 0% Ph+ metaphases) at 3 and 6 months. These landmark responses were correlated with long-term outcomes; namely, PFS, OS, and molecular response over time (MMR, MR4 [BCR-ABLIS ≤0.01%], MR4.5 [BCR-ABLIS ≤0.0032%]). The log-rank test was performed to compare pts who met the criteria for response versus pts who did not meet the criteria for response at the landmark with regard to PFS and OS. Data are as of Jan 6 2014. The median

follow-up was 27.9 (0.1-39.5) months. **Results**: 267 CP-CML pts were included in the analysis: 54% male; median age, 60 (18-94) years; median time from diagnosis, 7 (0.5-27) years. Pts were heavily pretreated: 60% received ≥3 TKIs. At 3 months, 51%, 36%, and 15% pts had reached ≤10% BCR-ABLIS, ≤1% BCR-ABLIS, and MMR, respectively. Pts who achieved each of these responses at 3 months were significantly more likely to have improved PFS after 2 years compared with those who did not (Table). The trend was similar for OS: specifically, pts who reached ≤1% BCR-ABLIS or MMR at 3 months had a significantly increased likelihood of OS after 2 years. Moreover, 3-month BCR−ABLIS levels correlated with achievement of deeper molecular response (MR4 and MR4.5) over time: pts with low BCR-ABLIS levels (≤1%) were more likely to achieve MR4 or MR4.5 compared with those having higher BCR-ABLIS levels (>10%). Furthermore, pts who achieved MCyR or CCyR at 3 months were significantly more likely to have improved PFS after 2 years compared with those who did not. This trend was similar for OS: achievement of MCyR or CCyR at 3 months was associated with an increased likelihood of OS after 2 years (Table). Similar trends were observed for 6-month molecular and cytogenetic landmark analyses (Table). **Conclusions**: In these refractory CML pts, the rapid and deep reduction in BCR−ABL levels achieved with ponatinib translated into improved long-term outcomes. These data validate the usefulness of assessing BCR-ABL levels at early time points as a goal of therapy with ponatinib since achieving early landmark response appears to be a strong predictor of better long-term outcomes.

[519] EPIC: A Phase 3 Trial of Ponatinib Compared with Imatinib in Patients with Newly Diagnosed Chronic Myeloid Leukemia in Chronic Phase (CP-CML). *Lipton.* **Background**: Ponatinib is an approved potent oral tyrosine kinase inhibitor active against native and mutated forms of BCR-ABL, including T315I. The phase 2 PACE study demonstrated that ponatinib is highly active in heavily pretreated Philadelphia chromosome–positive leukemia patients. Ponatinib efficacy and safety were evaluated in newly diagnosed CP-CML patients in the EPIC trial. **Methods**: EPIC was a multicenter, international, phase 3, randomized, 2-arm, open-label trial of ponatinib (45 mg once daily) compared with imatinib (400 mg once daily) in newly diagnosed CP-CML; patients were stratified by Sokal risk score (low [<0.8] vs intermediate [0.8 to ≤1.2] vs high [>1.2]). On 18 October 2013, EPIC was terminated due to the observation of arterial thrombotic events in the ponatinib development program. Consequently, none of the prospectively defined endpoints could be analyzed. Data as of 1 April 2014 are presented for endpoints that could be analyzed: BCR-ABLIS <10% rate at 3 months; major molecular response (MMR), molecular response (MR)4, and MR4.5 rates at and after at least 3, 6, 9, and 12 months and at any time; time to response; complete cytogenetic response rates at 6 and 12 months and any time; and safety. **Results**: At the time of study termination, 307 patients had been randomized; median follow-up was 5.1 (0.03-17.6) months. Groups were well-balanced with respect to sex, age, pretreatment, and Sokal score; however, the proportion of patients with 1 or more cardiovascular risk factors (hypertension, hypercholesterolaemia, diabetes, obesity and smoking) was higher in the ponatinib arm (n=97, 63%) compared to the imatinib arm (n=79, 52%). Data were available on 306 treated patients (154 ponatinib, 152 imatinib). Fourteen ponatinib and 2 imatinib patients discontinued due to adverse events (AEs). Molecular response rates for ponatinib were uniformly higher compared with imatinib for all response measures and at all time points (Table). The percentage of patients who achieved <10% BCR-ABL at 3 months was significantly higher in the ponatinib compared with imatinib arm overall (Table), and when patients were stratified by high-risk, intermediate-risk, and low-risk Sokal score (Figure). The percentage of patients who achieved MMR, MR4, and MR4.5 at any time in all Sokal risk groups was higher for ponatinib than imatinib (Figure). The most common (≥25%) all-grade treatment-emergent AEs with ponatinib were rash (38%), abdominal pain (36%), headache (33%), constipation (27%), increased lipase (27%), myalgia (26%), and thrombocytopenia (25%); with imatinib, they were nausea (34%), muscle spasms (34%), and diarrhea (27%). Twelve percent of ponatinib and 7% of imatinib patients had grade 3/4 thrombocytopenia; 3% of ponatinib and 8% of imatinib patients had grade 3/4 neutropenia. Serious treatment-emergent AEs (SAEs) occurring in ≥3 ponatinib patients were pancreatitis (n=5), atrial fibrillation (n=3), and thrombocytopenia (n=3); no individual SAEs occurred in ≥3 imatinib patients. Eleven (7%) ponatinib and 3 (2%) imatinib patients experienced arterial thrombotic events, designated serious for 10 [7%] ponatinib and 1 [0.7%] imatinib patient(s). One patient in the ponatinib arm experienced a serious venous thromboembolic event: there were none in the imatinib arm. Ten of 11 ponatinib patients, and 2 of 3 imatinib patients with arterial thrombotic events had 1 or more cardiovascular risk factors. **Conclusions**: Despite early termination, at a median follow-up of 5 months, preliminary evidence suggests that ponatinib has improved efficacy over imatinib in newly diagnosed CP-CML patients, but has a higher AE rate, including ATEs at the dose studied. Future investigations of ponatinib in the frontline setting will likely use lower doses and account for relevant risk factors.

[520] Seven-Year (yr) Follow-up of Patients (pts) with Imatinib-Resistant or -Intolerant Chronic-Phase Chronic Myeloid Leukemia (CML-CP) Receiving Dasatinib in Study CA180-034, Final Study Results. *Shah.* **Background**: Dasatinib is a potent BCR-ABL tyrosine kinase inhibitor (TKI) currently approved at 100 mg once daily (QD) as a first-line therapy in CML-CP pts and a second-line therapy in pts with CML resistant/intolerant to prior therapy. CA180-034 (NCT00123474), a prospective, randomized phase 3 study,

was designed to compare the dose and schedule of dasatinib therapy for the optimal benefit/risk ratio among pts with imatinib-resistant or -intolerant CML-CP. Results from this study have previously demonstrated significant efficacy of dasatinib in this pt population. Here, we report the final 7-yr analysis of efficacy and safety outcomes of CA180-034, which represents the longest follow-up of any second-generation BCR-ABL TKI to date. **Methods**: The CA180-034 2 X 2 factorial study design has previously been described (Shah 2010, *J Clin Oncol*). Pts (n=670) were randomized to dasatinib: 100 mg QD (n=167), 50 mg twice daily (BID; n=168), 140 mg QD (n=167), or 70 mg BID (n=168). To manage inadequate response or adverse events (AEs), dose escalation (up to a total daily dose [TDD] of 180 mg) and dose interruption or reduction (down to a TDD of 20 mg) were allowed. After 2 yrs, the protocol was amended to allow switching to a QD regimen with the same TDD after at least one dose reduction for recurrent anemia, thrombocytopenia, neutropenia, pleural effusion, or any other fluid retention during study progress or at the investigator's discretion (Shah 2014, *Blood*). **Results**: Approximately 55% (50 mg BID) and 51% (70 mg BID) of pts treated after the protocol amendment switched to QD dosing by the last recorded dose. The overall median duration of therapy was longer for the 100 mg QD group (37.4 months [mos]) compared with the 50 mg BID, 140 mg QD, and 70 mg BID groups (28.1 mos, 26.6 mos, and 28.9 mos, respectively). At 7 yrs of follow-up, progression-free survival (PFS) and overall survival (OS) rates were similar for all doses, as were the proportions of pts with a best on-study molecular response of MMR (Table 1). In an exploratory landmark analysis, pts in the 100 mg QD arm with BCR-ABL ≤10% (on the International Scale) at 3 mos had improved PFS and OS rates at 7 yrs relative to pts with BCR-ABL>10% (Table 2). BCR-ABL mutations were assessed in pts prior to the start of dasatinib (baseline), at the time of disease progression, or at end of treatment. Three mutations persisted or developed in pts who discontinued dasatinib due to loss of response on 100 mg QD: V299L (n=3), T315I (n=6), and F317L (n=7). For 100 mg QD, most nonhematologic and hematologic AEs (all grades) typically first occurred within the first 24 mos of treatment. Rates of nonhematologic AEs (all grades) over 7 yrs for 100 mg QD compared with other treatment arms included fluid retention (51% vs 54%), diarrhea (42% vs 47%), nausea/vomiting (27% vs 43%), myalgias/arthralgias (38% vs 33%), fatigue (37% vs 34%), and rash (33% vs 36%). Within yr 7 of the study, new cases of pleural effusion occurred in 5% (2/42) of pts at risk treated with dasatinib 100 mg QD compared with 8% (7/88) in other treatment arms. Severe (grade 3–4) AEs (any relationship) occurred less frequently in the 100 mg QD group (98/165, 59%) relative to other treatment arms (341/497, 69%). Three pts died due to study drug toxicity (1 due to sepsis; 1 due to pulmonary edema, congestive heart failure, neck pain, and pleural effusion; 1 due to necrosis of the colon). **Conclusions**: Long-term follow-up of dasatinib continues to demonstrate durable efficacy and benefit for pts with CML-CP following imatinib therapy, particularly if achieving BCR-ABL ≤10% at 3 mos. Dasatinib is well-tolerated amongst pts, with most AEs occurring early on during the course of treatment; however, pleural effusion did occur through 7 yrs of treatment. No new safety signals were detected.

[521] The Experience of the International Registry for Chronic Myeloid Leukemia (CML) in Children and Adolescents *(I-CML-Ped Study):* Pronostic Consideration *Millot.* **Aims**: An international registry (I-CML-Ped Study) was established to assess epidemiology, management and outcome of CML in the pediatric population. **Methods**: All national pediatric study groups were invited to include newly diagnosed children and adolescents less than 18 years with CML diagnosed later than January 2000. **Results**: Since January 2011, 351 patients from 12 countries have been registered. Clinical and biological data at initial diagnosis are available in 278 patients.There was a male preponderance (57%). The median age at diagnosis was 12.4 years (range, 9 months -17.5 years); 6% of the patients were younger than 4 years. At time of diagnosis 92% of the children were in chronic phase, 8% in accelerated phase and 1% in blastic phase according to the European Leukemia Net criteria. The Sokal risk group distribution was: 18% low, 31% intermediate and 51% high risk. The majority of the patients showed a Lansky score of 100 (59%) or 90 (21%). Splenomegaly was present in 76% of patients. The median of the spleen size below the costal margin was 11 cm (range: 1 to 25 cm). The median of the leukocyte count was 235x109/L (range: 5 to 1038). Additional chromosomal abnormalities in Ph-positive cells were observed in 6 % of the patients. The BCR-ABL transcript type was available in 227 patients: b3a2 54%, b2a2 38%, b3a2-b2a2 6% and b2a3 2%. The median follow-up time is 39 months (range, 0.5-161). Eight deaths were recorded. The estimated overall survival rate at 60 months is 95% (95%CI 89-97). Irrespective of treatment and follow-up, 124 (73%) of 169 assessable patients for cytogenetic response achieved complete cytogenetic response (CCyR). Exploratory analyses were performed in newly diagnosed patients regarding clinical and biological factors influencing the achievement of CCyR 12 months after starting imatinib. In univariate analyses, the Eutos score, the spleen size, the hematocrit level, the lymphocyte count and immature cells in peripheral blood, the percentage of granulocytes and monocytes in the marrow were identified as potential prognostic factors. However, only the percentage of the granulocytes in the marrow was identified as independent factor of achievement of CCyR at 12 months in multivariate analysis. Data collection and quality control regarding molecular assessment are ongoing. **Conclusion**: The data indicates that children and adolescents with CML presented with clinical and biological differences compared to adult patients with CML. Identification of prognostic factors is needed to optimize the strategy in the pediatric population.

[522] Impairment of Longitudinal Growth By Tyrosine Kinase Inhibitor (TKI) Treatment - Data from a Large Pediatric Cohort with Chronic Myeloid Leukemia (CML) *Tauer.* **Objectives**: A decade after being licensed for treatment of CML in minors, the TKI imatinib (IMA) is well known for it's inhibitory "off-target" effects on activity and proliferative capacity of osteoclasts and osteoblasts resulting in impaired bone remodeling (Vandyke K et al 2010 Blood 115:766; Tauer JT *et al* Blood 2011:118). This causes longitudinal growth retardation in not outgrown individuals (Millot F et al 2009 Blood 114:863; Shima H et al 2011 Pediatrics 159:676; Bansal D et al 2012 Ped Blood Cancer 59:481) which can be aggravated by a disrupted growth hormone:IGF-I axis as a possible additional off-target effect exerted by TKI treatment (Ulmer A et al 2013 Klin Padiatr 225:120; Bansal D et al 2012 Ped Blood Cancer 59:481). Starting a pediatric trial in the year 2006 which recruits approx. 15 pediatric patients (pts) with CML annually, we investigated to what extend growth is impaired depending on sex, age, and pubertal stage at start of IMA treatment in a pediatric cohort. **Methods**: 102 pts (54 male / 48 female; median age 12 years, range: 1-18 years) at diagnosis of CML receiving IMA as upfront treatment were enrolled retrospectively in this analysis from centers in Germany and participating countries during 02/2006 to 06/2014. Height standard deviation scores (SDS) were derived from WHO-AnthroPlus, version 1.04 software, a global growth-monitoring tool providing normal range values for the age cohorts from birth till 19 years. 81 out of 102 pts fulfilled the criteria for continuous assessment of growth scheduled at three months intervals during IMA exposure. 21 pts were analyzed at intervals ≠ 3 month. Pts excluded comprised individuals shifted to a 2nd generation TKI, or cumulative interruptions of drug intake exceeding 4 weeks, or pts undergoing stem cell transplantation. **Results**: The mean and median duration of IMA exposure was 12 months and 9 months, respectively (range: 0–98 month). 27/102 pts (13 male, 14 female) were prepubertal (age: <10 years) at initiation of IMA treatment while 46/102 pts were pubertal (age: 10-14 years; 23 male, 23 female), and 29/102 pts were in postpubertal stage (age: >14 years; 18 male, 11 female). In comparison to mean SDS at diagnosis a mean decrease in height of 0.48 SDS per year was observed in the total cohort during the first three years of treatment, being more pronounced in prepubertal pts. In pts diagnosed shortly before or at puberty a mean reduction of 0.75 SDS per year during the first three years were observed. Older teenagers revealed no change in body height z-score during TKI treatment compared to height z-score at diagnosis. **Discussion**: Growth retardation is a significant adverse effect of IMA in children with CML affecting predominantly prepubertal children. Possible medical interventions still need to be investigated.

6 TKI cessation, monitoring and resistance [811-816]

[811] Dasatinib or Nilotinib Discontinuation in Chronic Phase (CP)-Chronic Myeloid Leukemia (CML) Patients (pts) with Durably Undetectable *BCR-ABL* Transcripts: Interim Analysis of the STOP 2G-TKI Study with a Minimum Follow-up of 12 Months – on Behalf of the French CML Group Filmc. *Rea.* **Background**:Tyrosine kinase inhibitors (TKIs) targeting BCR-ABL have revolutionized the prognosis of pts suffering from CML but these drugs are considered as non-definitively curative and current recommendation is to treat /ggest that imatinib may be successfully stopped in pts with deep and sustained molecular responses. Here, we report on the feasibility of second generation TKIs discontinuation in the setting of the French STOP 2G-TKI study. **Methods**: Adult CP-CML pts on dasatinib or nilotinib first line or after imatinib without prior allogeneic transplantation or progression to advanced phase CML were proposed TKI discontinuation when presenting: (1) b2a2 or b3a2 *BCR-ABL* transcripts subtype, 2) TKI treatment duration for at least 36 months, (3) CMR4.5 achieved and maintained for at least 24 months. The primary objective was treatment-free survival without loss of major molecular response (MMR). After TKI discontinuation, *BCR-ABL* transcripts were monitored monthly during the first 12 months, every 3 months during the 2ndyear and every 3 to 6 months thereafter. Molecular relapse was defined by MMR loss on a single occasion and triggered TKI reintroduction. Data as of August 1, 2014 are reported in pts with at least 12 months of follow-up (n=52) and median follow-up was 32 months (12-56). **Results**: Median age was 60 years (34-81) and 61.5% of pts were female. Sokal risk group was low in 58%, intermediate in 23%, high in 13% and unknown in 6%. 2G-TKIs were given after imatinib intolerance in 67% of pts, suboptimal response or resistance to imatinib in 23% and upfront in 10%. Median duration of CML, TKI treatment, 2G-TKI treatment and CMR4.5 was 83 months (36-218), 78 months (36-136), 39 months (19-72) and 28 months (24-64), respectively. Twenty four pts lost MMR after a median time of 4 months (1-38) at last follow-up. Importantly, no loss of CHR or progression to advanced phase CML was observed. The 12- and 24-month probabilities of treatment-free survival without MMR loss were 61.4% (95% CI, 48.1-74.6) and 57% (95% CI, 43.3-70.6), respectively. The majority of relapses occurred within 6 months and in a landmark analysis, pts who were still in MMR without therapy at 6 months had 12- and 24-month probabilities of treatment-free survival without MMR loss of 91.2% (95% CI, 81.6-100) and 84.7% (95% CI, 72.2-97.1), respectively. All pts but 1 who lost MMR restarted 2G-TKI treatment and regained MMR after a median time of 3 months (1-8). Pts in MMR without any therapy (n=28) displayed varying patterns of spontaneous molecular response including stable CMR4.5 in 7 and fluctuations between CMR4.5 and MR4.5, CMR4.5 and MR4, CMR4.5 and MMR in 9, 4 and 4 pts, respectively. Gender, age, prior interferon exposure, 2G-TKI type, treatment duration and duration of CMR4.5 were not found to

have any impact on outcome. By contrast, prior history of suboptimal response or resistance to imatinib was associated with a significantly lower chance of successful treatment discontinuation, with a 12-month probability of treatment-free survival without MMR loss of 41.7% (95% CI; 13.8%-69.6%), compared to 67.3% (95% CI, 52.6%-81.8%) in other patients (p=0.04). **Conclusions**: 2G-TKI could be safely and successfully discontinued in CP-CML pts with long-lasting undetectable *BCR-ABL* transcripts, especially in those without prior history of suboptimal response or resistance. Most of molecular relapses had an early onset and all were sensitive to 2G-TKI resumption. The recurrence of low levels of detectable residual disease below MMR after 2G-TKI withdrawal did not automatically herald CML relapse and did not preclude the possibility to remain treatment-free.

[812] Early Disease Relapse after Tyrosine Kinase Inhibitor Treatment Discontinuation in CML Is Related Both to Low Number and Impaired Function of NK-Cells. *Ilander.* **Background**: Recent reports suggest that approximately 40% of CML patients who have achieved sustained complete molecular remission are able to stop TKI treatment without disease relapse. However, there are no predictive markers for successful therapy discontinuation. Therefore, we set up an immunological sub-study in the ongoing pan-European EURO-SKI stopping study. Our aim was to identify predictive biomarkers for relapse/non-relapse and to understand more on the mechanisms of immune surveillance in CML. **Methods**: The EURO-SKI study started in 2012, and patients included were at least three years on TKI and at least one year in MR4 or deeper before the study entry. Basic lymphocyte immunophenotyping (the number of NK-, T- and B-cells) was performed at the time of therapy discontinuation and 1, 6, and 12 months after the TKI stop and in case of relapse (defined as loss of MMR, *BCR-ABL1*>0.1% IS). In addition, from a proportion of patients more detailed immunophenotypic and functional analyses (cytotoxicity of NK-cells and secretion of Th1 type of cytokines IFN-γ/TNF-α) were done at the same times. **Results**: Thus far 119 Nordic patients (imatinib n=105, dasatinib n=12, nilotinib n=2) who have discontinued TKI treatment within the EURO-SKI study have been included in the lymphocyte subclass analysis (results are presented from patients who have reached 6 months follow-up). Immunophenotyping analysis demonstrates that imatinib treated patients who were able to maintain remission for 6 months (n=36) had increased NK-cell counts (0.26 vs. 0.15x109cells/L, p=0.01, NK-cell proportion 18.9% vs. 11%, p=0.005) at the time of drug discontinuation compared to patients who relapsed early (before 5 months n=22). Furthermore, the phenotype of NK-cells was more cytotoxic (more CD57+ and CD16+cells and less CD62L+cells), and also their IFN-γ/TNF-α secretion was enhanced (19.2% vs. 13%, p=0.02). Surprisingly, patients who relapsed more slowly (after 5 months, n=16) had similar baseline NK-cell counts (0.37x109cells/L), NK-cell proportion (21.2%), and phenotype and function as patients, who were able to stay in remission. No differences in the NK-cell counts were observed between patients who had detectable or undetectable *BCR-ABL1* transcripts at the baseline (0.22 x109cells/L vs. 0.31 x109cells/L, p=0.61). Interestingly, NK-cell count was higher in patients with low Sokal risk score than in patients with intermediate risk (0.33 x109cells/L vs. 0.20 x109cells/L, p=0.04). Furthermore, there was a trend that male patients had a higher proportion of NK-cells than females (21.6% vs. 15.7%, p=0.06). Pretreatment with IFN-α or the duration of imatinib treatment did not have an effect on NK-cell count or proportion. In comparison to the imatinib group, dasatinib treated patients had higher NK-cell counts at the baseline (median 0.52x109cells/L vs. 0.26x109cells/L, p=0.02), and also the proportion of CD27 (median 50% vs. 16%, p=0.01) and CD57 expressing (median 79% vs. 74%, p=0.05) NK-cells was higher. The follow-up time of dasatinib treated patients is not yet long enough to correlate the NK-cell counts with the success of the treatment discontinuation. The absolute number of T-cells or their function did not differ significantly between relapsing and non-relapsing patients at the time of treatment discontinuation. However, both CD4+ and CD8+ T-cells tended to be more mature in patients who stayed in remission compared to patients who relapsed early (CD4+CD57+CD62L- median 5.7% vs. 2.4%, p=0.06, CD8+CD62L+CD45RA+ 13% vs. 26.7%, p=0.05). The analysis of follow-up samples showed that in patients who stayed in remission the Th1 type cytokine (IFN-γ/TNF-α) secretion of CD8+T-cells increased at 6 months compared to baseline (23.6 vs. 18.5%, p=0.07). Same phenomenon was observed in the late relapsing group at relapse compared to baseline (37.9 vs. 13.5%, p=0.03). No similar increase was observed in the early relapsing group. **Conclusions**: Low NK-cell numbers and poor cytokine secretion may predict early disease relapse after TKI discontinuation. However, patients who relapse later have high numbers of normally functioning NK-cells. Further research (detailed phenotypic analysis of NK- and T-cells including activating and inhibitory receptors and immune checkpoint molecules) and correlation of biomarker data with clinical parameters are ongoing to understand the ultimate determining factors of relapse.

[813] The Risk of Relapse in CML Patients Who Discontinued imatinib Can Be Predicted Based on Patients Age and the Results of dPCR Analysis. *Mori.* **Introduction**. Chronic myeloid leukemia (CML) patients (pts) treated with imatinib first line achieve complete cytogenetic response (CCyR) in > 70% of cases and major molecular response (MMR) in 18-58%. These pts have a life expectancy similar to the general population. However even undetectable BCR-ABL may not equate to eradication of the disease because of the sensitivity of Q-RT-PCR. A new diagnostic method, the digital-PCR (dPCR), able to detect 1 BCR-ABL+ cell out of 107 cells, has been recently developed (Goh HG et al., 2011). dPCR corresponds to a 100 fold

increase in sensitivity as compared to Q-RT-PCR. Therefore, dPCR by assessing the presence of minimal residual disease with higher sensitivity, could potentially identify pts in whom CML has been eradicated. **Aims**. The Imatinib Suspension And Validation (ISAV) study is aimed at validating the capability of dPCR to predict relapses after imatinib discontinuation in CML pts with negative Q-RT-PCR results. **Methods**. This study involves 15 sites, 10 in Italy and 1 in each of the following countries: Germany, Spain, The Netherlands, Canada and Israel. CML pts (Chronic or Accelerated Phase) under imatinib therapy since more than 2 years and in complete molecular remission (CMR) were eligible for this study. Patients had to be in CMR for at least 18 months (mts), with a minimum of 3 Q-RT-PCR performed at their own sites. After signing the informed consent, blood samples are obtained for dPCR and the pts discontinue imatinib therapy. Standard Q-RT-PCR is performed monthly (mts 1-6) and then bimonthly for 36 mts to assess the maintenance of the molecular remission. The loss of molecular remission is defined as two consecutive positive Q-RT-PCR tests with at least one BCR-ABL/ABL value above 0.1%. Patients losing molecular remission resume imatinib treatment at the same dosage used before interruption. Patients' quality of life during imatinib discontinuation/resumption is evaluated through the EORTC – C30 Quality of Life questionnaire. **Results**. The enrolment in ISAV began in November 2011 and ended in July 2013. The study enrolled 112 pts: Italy 69.6%, Germany 21.4%, Canada 5.3%, Spain 2.6% and Israel 0.9%. Among the 112 pts, 59.3% were male and 37.0% were aged 65 or older; median duration of imatinib treatment was 103.1 mts with median duration of CMR of 25.8 mts before imatinib discontinuation. To date, the median follow-up (FUP) time is 16.6 mts [95% CI: 14.9-18.2]. Forty-seven pts (43.5%, 95% CI: 34.0-53.4) of the 108 eligible pts relapsed and resumed imatinib; 38/47 (80.9%) of them relapsed in the first 9 mts and the last relapse occurred 19.6 mts after imatinib discontinuation. A loss of CCyR occurred in 11 pts (23.4%): 10/11 CCyR losses were recovered; 1 patient withdrew the consent shortly after obtaining a partial cytogenetic response. No case of CML progression was observed. After the resumption of imatinib the median time to either MMR or CMR was 1.9 [95% CI: 1.2-2.4] mts. Of the 61 not-relapsed pts, 43 (39.8% of the total) regained Q-RT-PCR positivity but never lost MMR. The median time to Q-RT-PCR positivity was 3.6 mts [95% CI: 3.0-4.8] and the range of duration of Q-RT-PCR positivity (below 0.1%) was between 5.7 and 29.2 mts. No significant correlation between relapse and previous duration of imatinib treatment, use of interferon, time to CCyR or duration of CMR was identified. An inverse relationship between pts age and risk of relapse is evident: 90% of pts < 45 years relapsed vs 37.5% in the class ≥ 45 - < 65 years and 27.5% of pts ≥ 65 years, p(χ2)<0.0001. dPCR results showed that 23.4% of pts were positive and 76.6% negative, with a dPCR Negative Predictive Value (NPV) of 63.4% (Tab.1) and a significant NPV ratio (dPCR/Q-RT-PCR) of 1.131 [95% CI: 1.032-1.239]. Age and dPCR results predicted the risk of relapse: pts with less than 45 years and with a positive dPCR had the highest risk of relapse (100%) as opposed to pts ≥ 45 years and with negative dPCR (30.6%; Fig.1). **Conclusions**. After 32 mts from the beginning of the study, with a median FUP of 16.6 mts, 43.5% of pts relapsed; the majority of relapses developed in the first 9 months after imatinib discontinuation. Age < 45 years and dPCR positivity are significantly associated with relapses.

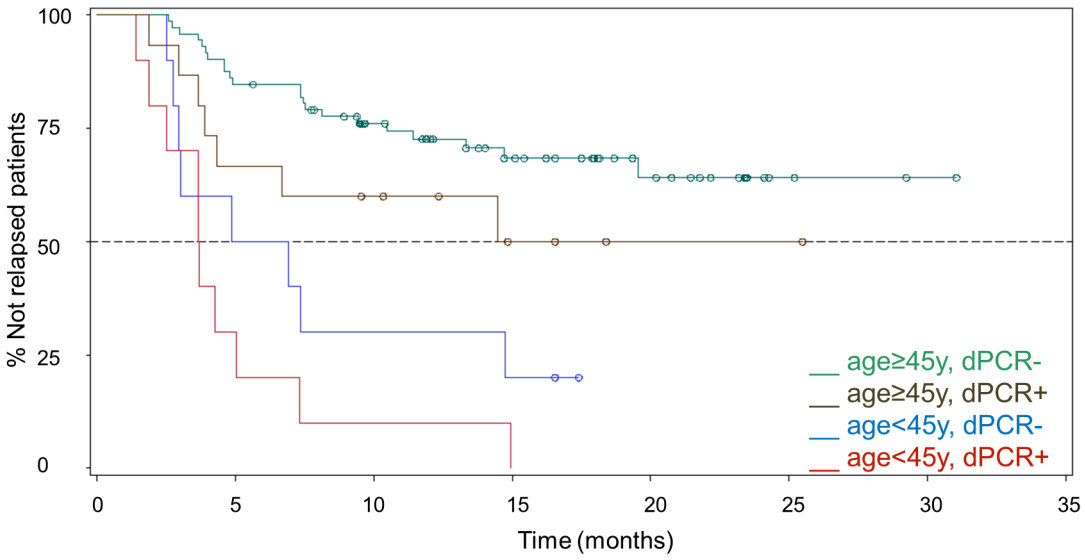


Fig 1. Age and dPCR correlation (Kaplan-Meier Curve)

[814] KIR2DL5B Genotype Independently Predicts Poor Outcomes in CML-CP Patients Switched to Nilotinib after Suboptimal Responses to Imatinib and May Refine Prognosis in Patients with EMR Failure. *Yeung.* **Introduction**. A correlation exists between innate immune responses and outcomes in cancer

treatment, and immunological features may be prognostic biomarkers of TKI response in chronic phase CML patients (CML-CP pts). Marin et al (*Leuk* 2012) found KIR2DS1 to be associated with CCyR, OS and PFS, while Kreutzman et al (*Exp Hem* 2012) showed KIR2DL5A/B to be associated with MMR. We examined the prognostic significance of KIR (Killer Immunoglobulin-like Receptor) genotypes in CML-CP pts in the TIDEL-II study who received upfront treatment with imatinib and early switching to nilotinib for suboptimal responses. **Method.** TIDEL-II is a multicentre, single arm prospective ALLG trial for de novo CP-CML pts in 2 equal sequential cohorts of 105 pts in each. All pts started on imatinib (IM) 600mg OD. Pts were monitored for achievement of time dependent molecular targets (BCR-ABL 10%, 1% and 0.1% IS at 3, 6 and 12 months respectively). Pts in cohort I (C1) who failed these targets were dose escalated to IM800. Pts failing to achieve these targets subsequently, or who were already on IM800, switched to nilotinib 400mg BID (NIL). Pts in cohort II (C2) who failed their time dependent targets switched to NIL regardless of their IM dose. Switching to NIL was also permitted for Grade III/IV or persistent Grade II non-hematological toxicity or loss of response. Baseline samples were available for 148 pts, on which KIR genotyping was done retrospectively using the KIR Genotyping SSP Kit (Invitrogen, Carlsbad, CA). Molecular response and survival outcomes were analysed as stratified by early molecular response (EMR, BCR-ABL ≤10% at 3 months), gender, Sokal Index, age and KIR genotype. **Results.** The 24 month MMR rate was 73% and EMR failure was 12%. Overall and progression-free survival (PFS; events = transformation to AP/BC + any death) was 94% and 93% at 4 years respectively. Failure free survival (FFS; events in PFS in addition to loss of MMR / CCyR and failure according to 2013 ELN criteria) was 76% at 4 years. In a competing risk univariate analysis, EMR correlated with MMR achievement as expected, but not Sokal, age or sex. This analysis also showed inferior MMR achievement for pts with KIR2DL5B (HR 0.423, p=0.00041), KIR2DL2 (HR 0.607, p=0.0048) and KIR2DS3 (HR 0.547, p=0.0027) genotypes. The number of pts with these alleles were 31 (21%), 83 (56%) and 44 (30%) respectively. As predicted from the population distribution of KIR genotypes, these 3 alleles were in strong linkage disequilibrium. Only KIR2DL5B (2DL5B) and EMR remained independent predictors of MMR in a multivariate model (2DL5B positive HR 0.52, p=0.034). KIR2DL5B positivity was also independently associated with inferior rates of MR4.5 (HR 0.42, p=0.031) (Fig. top panels). Four year PFS was inferior for pts positive for 2DL5B (86% vs 97%, p=0.04), as was FFS (67% vs 80%, p=0.05) (Fig. lower panels). There was no correlation between 2DL5B status and EMR achievement. However pts negative for 2DL5B who had EMR failure were more likely to achieve MMR, 7/13 pts (54%), compared with 0/5 2DL5B positive pts (all 18 pts switched to NIL). This was not statistically significant (p=0.09), due to the small numbers. In patients who achieved EMR, 94% of 100 2DL5B negative pts achieved MMR, vs 76% of the 25 2DL5B positive pts. Among pts with EMR failure, 2DL5B positivity was associated with a trend for inferior survival at 4 years. PFS was 91% vs 98% and FFS was 80 vs 84% for 2DL5B pos vs neg pts respectively. **Conclusion.** KIR genotypes had previously been correlated with achievement of CCyR, PFS and OS. Here, we have demonstrated that the KIR2DL5B allele correlated with lower rates of MMR and MR4.5 in a multivariate analysis, even in a treatment schema allowing patients failing early molecular targets to be treated with nilotinib, independent of EMR. Additionally, KIR2DL5B positivity was associated with inferior PFS and FFS. Multiple studies have shown the prognostic significance of EMR, as has our data. KIR genotyping information may further refine the prognosis of patients failing to achieve EMR. Prognostic markers available at CML-CP diagnosis, such as KIR genotypes, may have clinical utility. Furthermore, the KIR genotype may provide useful information in combination with other biomarkers and could be incorporated into future prognostic scoring systems for CML-CP.

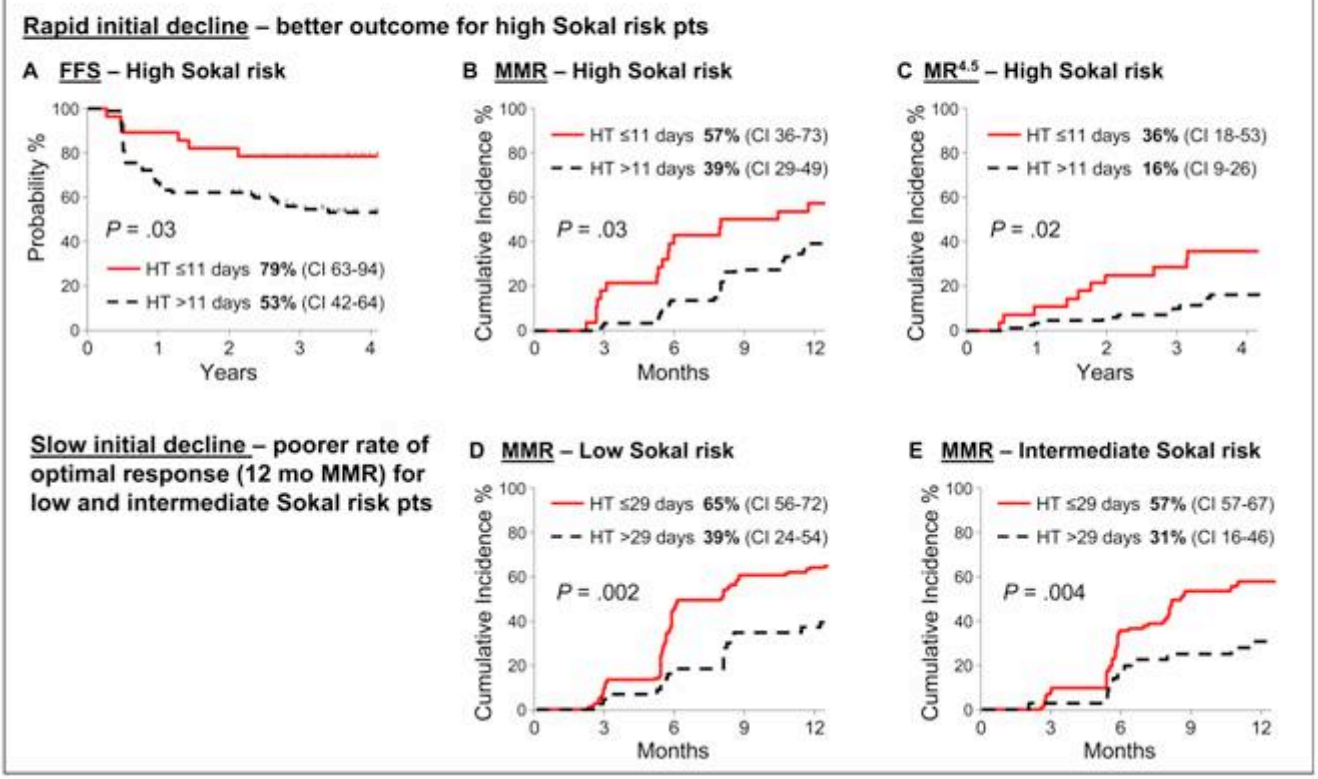
[815] In Chronic Myeloid Leukemia Patients on 2nd-Line Tyrosine Kinase Inhibitor Therapy, Deep Sequencing at the Time of Warning May Allow Sensitive Detection of Emerging BCR-ABL1 Mutants. *Soverini.* **Background and Aims:** Next generation amplicon-based deep sequencing (DS) on the Roche, Illumina or Ion Torrent instruments is becoming accessible to a wider and wider number of diagnostic laboratories. Although conventional sequencing is still the gold standard, DS has been hailed by many as the future of diagnostic BCR-ABL1 kinase domain (KD) mutation screening. BCR-ABL1 KD mutations are infrequent in newly diagnosed chronic myeloid leukemia (CML) patients (pts) receiving 1st-line TKI therapy, but remain a challenge in relapsed pts, who usually display a greater genetic instability. Indeed, pts already harboring BCR-ABL1 KD mutations have a higher likelihood of developing additional, dasatinib (DAS)- or nilotinib (NIL)-resistant mutations – which is defined as a 'failure' by the 2013 European LeukemiaNet (ELN) recommendations. Taking advantage of a next-generation amplicon sequencing design and protocol set up and validated in the framework of the IRON-II international study, we aimed to assess whether DS may allow a larger window of detection of emerging BCR-ABL1 KD mutants predicting for an impending relapse. **Methods**: among the imatinib (IM)-resistant CML pts who switched to 2nd-line TKI therapy and were referred to our laboratory for routine BCR-ABL1 transcript level monitoring and KD mutation screening by conventional sequencing, 51 acquired DAS- or NIL-resistant mutations after a median of 9 months (range, 3-27 months) of therapy and had leftover cDNA available at previous timepoints. To reconstruct the dynamics of mutation emergence, resequencing on a Roche GS Junior instrument was performed from the time of failure and mutation detection by conventional sequencing backwards. Runs were designed to achieve high sequencing depth, allowing reliable detection of variants down to 1% abundance. BCR-ABL1/ABL1%IS transcript levels and/or cytogenetic response, whichever available, were used to define whether the patient had an 'optimal

response', 'warning' or 'failure' at the time of first mutation detection by DS. **Results**: baseline mutation status, as assessed by conventional sequencing, was available for all the 51 CML pts included in this retrospective study; 29/51 pts were positive for BCR-ABL1 KD mutations, with switch to NIL or DAS selected accordingly. Twenty-six pts were later found to have acquired DAS-resistant mutations (T315I, n=13; F317L/V, n=10; V299L, n=3) and 25 pts were later found to have acquired NIL-resistant mutations (T315I, n=4; F359V/I/C, n=7; Y253H, n=6; E255K, n=9; one patient acquired two mutations). DS was able to backtrack the DAS- or NIL-resistant mutations to the previous sample(s) in 23/51 (45%) pts. Median mutation burden at the time of first detection by DS was 5% (range, 1-17%); median interval between detection by DS and detection by conventional sequencing was 3 months (range, 3-9 months). In 5 cases, the mutations were traceable at baseline; in the remaining cases, correlation with response at the time mutations were first detected by DS revealed a 'warning' according to the 2013 ELN definitions of response to 2nd-line therapy in 13 cases; an 'optimal response' in one case; a 'failure' in 4 cases. As a control, we used DS to explore BCR-ABL1 KD mutation status in 10 randomly selected pts with 'warning' at various timepoints, that later turned into optimal responses; no DAS- or NIL-resistant mutations were detected. **Conclusions**: the 2011 ELN recommendations for mutation analysis suggest BCR-ABL1 KD to be screened by conventional sequencing in case of 'failure' of 2nd-line TKI therapy – according to the provisional definitions available at the time. Earlier detection of emerging BCR-ABL1 KD mutations allows a greater leeway in tackling drug resistance and enhancing therapeutic efficacy. Data presented herein indicate that: 1) DS may reliably pick TKI-resistant mutations earlier than conventional sequencing in a proportion of pts, and that 2) the recently introduced definitions of 'warning' may provide a rational trigger, besides 'failure', for DS-based BCR-ABL1 KD mutation screening in CML pts on 2nd-line TKI therapy. A prospective cost-benefits evaluation of using DS in this and other settings is warranted, and will contribute useful information to the revision of the ELN recommendations for BCR-ABL1 KD mutation analysis.

[816] The Adverse Effect of High Sokal Risk for First Line Imatinib Treated Patients Is Overcome By a Rapid Rate of BCR-ABL Decline Measured As Early As 1 Month of Treatment. *Branford.* **Background**: Clinical scoring systems, such as Sokal risk, continue to have prognostic relevance for patients (pts) treated with tyrosine kinase inhibitors (TKI) and may have utility in combination with emerging biomarkers. The BCR-ABL value at 3 and 6 months (mo) of TKI are the strongest predictors of response. However, recent data demonstrate that the rate of BCR-ABL decline from the pre imatinib level adds significant predictive information (Hanfstein, Leukemia 2014; Branford, Blood 2014). Among poor risk pts with >10% at 3 mo in our cohort of first line imatinib, those with a slow rate of decline from their pre imatinib value, assessed by calculating the number of days over which BCR-ABL halved (halving time), predicted significantly poorer outcomes. Notwithstanding the importance of the 3 and 6 mo values, a prognostic biomarker obtained at an earlier timepoint may allow opportunities for therapy optimization. We therefore examined the prognostic significance of the rate of BCR-ABL decline at 1 mo in the context of other predictors of response.**Aim**: To determine whether baseline factors (age, gender, Sokal risk and imatinib starting dose: 400, 600 or 800 mg) and the BCR-ABL halving time at 1 mo of imatinib have predictive significance. **Method**: 528 first line imatinib treated pts were evaluated (median 45 mo of imatinib). Molecular assessment was performed pre imatinib and at 1 mo for 470/528. 453 of these 470 pts had a Sokal score available and were included in the analysis of outcome. **Results**: The median BCR-ABL halving time at 1 mo of imatinib was 17 days, quartiles 11, 29. An initial rapid BCR-ABL decline, indicated by halving times in the lowest quartile of ≤11 days (n=115), was associated with significantly superior rates of MMR by 12 mo, MR4.5 and failure-free survival (FFS) by 4 years compared with longer halving times of >11 days (n=338), Table. MMR by 12 mo was assessed since it represents an optimal response and is associated with subsequent deep molecular response. By univariate and multivariate regression analysis only the 1 mo halving time and Sokal risk significantly predicted MMR, MR4.5 and FFS. These factors were independent and there was no difference between the median 1 mo halving times among the Sokal risk groups, P = .36. The high Sokal risk pts had significantly poorer outcomes. To improve response prediction, these pts were divided into 2 groups according to their 1 mo halving time; ≤11 days (n = 28) and >11 days (n=90). A 1 mo halving time of ≤11 days was associated with significantly improved outcomes for these pts, Table and Figure. The responses equated to those of pts with low Sokal risk: high risk ≤11 days vs low risk: MMR by 12 mo 57% vs 59%, P = .95; MR4.5 by 4 years 36% vs 40%, P = .82; FFS by 4 years 79% vs 84%, P = .39. The high Sokal risk pts with the rapid initial BCR-ABL decline also had a lower probability of BCR-ABL >10% at 3 mo (early molecular response [EMR] failure), which is considered a warning or treatment failure; ≤11 days vs >11 days: 14% vs 33%, Table. In contrast to the improved response observed with a rapid initial decline for high Sokal risk pts, a slow decline for pts with low or intermediate Sokal risk (halving times in the upper quartile of >29 days) was associated with a significantly lower cumulative incidence of MMR by 12 mo: low Sokal risk ≤29 days (n = 151) vs >29 days (n = 44) 65% vs 39%, P = .002; intermediate Sokal risk ≤29 days (n = 103) vs >29 days (n=37) 57% vs 31%, P = .004. **Conclusion**: Imatinib treated high Sokal risk pts have a higher rate of treatment failure and poorer molecular response. However, our data suggest their prognosis can be refined by taking into account the kinetics of BCR-ABL decline after only 1 mo of treatment. A rapid initial decline defined a subgroup of high Sokal risk pts with outcomes equivalent to those of low Sokal risk pts. Frequent molecular monitoring in the

critical first months of treatment could enhance outcome prediction and limit the indication for a change of treatment.

Figure. Response for Sokal risk groups according to the rate of BCR-ABL decline from the pre imatinib value when measured at 1 mo of imatinib. (A-C) For patients with high Sokal risk a rapid initial decline of BCR-ABL (halving time (HT) of ≤11 days) was associated with improved responses and outcome. (D and E) For patients with low or intermediate Sokal risk, a slow initial decline (>29 day HT) was associated with significantly lower MMR by 12 months. MMR and $MR^{4.5}$ were confirmed responses. Failure events included lack of ELN milestone responses, loss of any response, BCR-ABL mutations, clonal chromosomal abnormalities in Ph+ cells, AP/BC and death.



7 Biology and Pathophysiology, excluding Therapy: Poster I [1783-1791]

8 abstracts: https://ash.confex.com/ash/2014/webprogram/Session5801.html

1783. Comparative TKI Profiling Analyses to Explore Potential Mechanisms of Ponatinib-Associated Arterial Thrombotic Events
1784. Inhibitory Effects of Icaritin on TKI-Resistant CML Cells through Disruption of the BCR-ABL-GRB2-RAS-MAPK Signaling Pathway
1785. Connective Tissue Growth Factor Induces Anti-Apoptotic Factors in Chronic Myeloid Leukemia Stem Cells
1786. The Tumor Suppressors, MS4A3 and G0S2, Are Downregulated in CML Cells with BCR-ABL1 Kinase-Independent Resistance
1787. Expression of the ETS Transcription Factor GABPα Is Correlated to *BCR-ABL/ABL* ratio in Human CML and Mediates Imatinib Sensitivity
1788. High Plasma Levels of TGF-α and IL-6 at Diagnosis Predict Early Molecular Response Failure and Transformation in CML
1789. KLF4 Regulates Self-Renewal of Leukemic Stem Cells in Chronic Myeloid Leukemia By Repressing Gbl Expression and Altering mTORC2 Activity
1790. PKC Pathways Mediate BCR-ABL-Independent Imatinib Resistance in Chronic Myeloid Leukemia
1791. Chronic Myeloid Leukemia Stem Cells (LSCs) and Leukemia Progenitor Cells (LPCs) Display Overlapping and Unique Mechanisms of Genomic Instability: The Role of PI3k-AKT and PI3k-Rac2-PAK Pathways

8 Therapy: Poster I [1792-1817]

25 abstracts: https://ash.confex.com/ash/2014/webprogram/Session5802.html

1792. A Comparison of Droplet Digital PCR and Quantitative RT-PCR for Low Level BCR-ABL in CML Patients with Molecular Responses
1793. Long Term Outcome of Chronic Phase Chronic Myeloid Leukemia (CP CML) Patients (pts) from the French Spirit Study Comparing Imatinib (IM) 400 Mg to Higher Dose Imatinib or Combination with Peg-interferonα2a (PegIFN) or Cytarabine (Ara-C)
1794. Updating Long-Term Outcome of Intermittent Imatinib (INTERIM) Treatment in Elderly Patients with Ph+-CML
1795. A Shorter Halving Time for *BCR-ABL* transcript Reduction Is a Novel Predictor of Molecular Response Achievement in Newly Diagnosed Chronic-Phase Chronic Myeloid Leukemia (CML-CP) Patients Treated with Dasatinib: Results of D-First Study
1796. Deep Molecular Response in Patients With Newly Diagnosed Chronic Myeloid Leukemia in Chronic Phase (CML-CP) Treated With Nilotinib: ENESTnext Update
1797. *BCL2L11* (*BIM*) Deletion Polymorphism Is Associated with Molecular Relapse after ABL Tyrosine Kinase Inhibitor Discontinuation in Patients with Chronic Myeloid Leukemia with Complete Molecular Response
1798. Decisions Taken in Children and Adolescents with Chronic Myeloid Leukemia (CML) at Failure of Imatinib Treatment
1799. The Effect of Nilotinib in Chronic Myeloid Leukemia Treatment Dose on Spermatogenesis and Folliculogenesis in a Healthy Mouse Model
1800. Further Evaluation of Pro-Atherogenic and Anti-Angiogenic Effects of Nilotinib in Mice and in Patients with Ph-Chromosome+ CML
1801. Chronic Myeloid Leukemia in Chronic Phase: Survival in the Era of Tyrosine Kinase Inhibitors Is Similar to That of the General Population in All Age Groups
1802. Propensity Score Matched Comparison of Dasatinib and Nilotinib As a Frontline Therapy in Newly Diagnosed CML with Chronic Phase
1803. Characteristics and Outcomes of Unselected Adolescents and Young Adults Patients with Chronic Myeloid Leukemia in the Tyrosine Kinase Inhibitor Era
1804. The Use of Statin Enhances Chance of Achieving MR4.5 in Chronic Myeloid Leukemia Patients in Chronic Phase Following Imatinib Therapy
1805. Odk-1201, One-Step RT-qPCR Major *BCR-ABL/ABL* mRNA Kit for the International Scale, with High Sensitivity to Detect Deeper MR
1806. Gimema Registry of Conception/Pregnancy in Adult Patients Diagnosed with Chronic Myeloid Leukemia (CML) Treated with Tyrosine Kinase Inhibitors (TKIs)
1807. BCR-ABL Testing Frequency Lower Than NCCN Recommendations in Lab Network Review of CML Patients
1808. Medication Adherence in Patients with Chronic Myelogenous Leukemia Using Tyrosine-Kinase Inhibitors: A Retrospective Analysis
1809. Evaluation of Cepheid Xpert® BCR-ABL Monitor Assay in Three Italian Reference Centers for Monitoring of BCR-ABL Transcript Levels in CML Patients
1810. Detection of Compound BCR-ABL Mutations in TKI Resistant CML Patients Including a Novel K245N Mutation Associated with Primary Nilotinib Resistance By Employing a Newly Developed Cost Effective BCR-ABL Sequencing Protocol
1811. Arterial Thrombotic Complications Are Uncommon in Patients without Cardiovascular Risk Factors and Occur at Equivalent Rates in Chronic Myeloid Leukemia (CML) Patients Treated with Imatinib and Nilotinib
1812. Response to High Dose Imatinib and Long-Term Outcome in Children and Adolescents with Previously Untreated Chronic Myeloid Leukemia in Chronic Phase. the Italian Experience
1813. Comparison of Glucose and Lipid Metabolism Abnormality during Nilotinib, Imatinib and Dasatinib Therapy – Results of Enigma 2 Study
1814. Cytomegalovirus Infection Is Associated with Expansions of CD8 T Cells and Highly Oligoclonal Vdelta1 Gamma/Delta T Cells in Patients Treated with Dasatinib for Chronic Myelogenous Leukaemia
1815. Final Results From SENSOR: Switch to Nilotinib After Molecular Suboptimal Response (SoR) to Frontline Imatinib in Patients With Chronic Myeloid Leukemia in Chronic Phase (CML-CP)
1816. Efficacy of Nilotinib Vs High-Dose Imatinib Vs Sustaining Standard-Dose Imatinib in Early Chronic Phase CML Patients Who Have Suboptimal Molecular Response to Frontline Imatinib
1817. Potential of Digital PCR in CML Calibration

9 Biology and Pathophysiology, excluding Therapy: Poster II [3125-3133]

8 abstracts: https://ash.confex.com/ash/2014/webprogram/Session5968.html

3125. FOXM1 Transcription Factor Is a Component of Beta Catenin Signaling in Hematopoietic Progenitors of Chronic Myeloid Leukemia
3126. In CML Patients with Good Response to TKIs Other Gene Mutations Are Frequently (37%) Present in Addition to Philadelphia

Negative, Cytogenetically Aberrant Clones but Are Rare (4%) in Cases with MMR and Normal Karyotype
3127. Anti-Leukemic Activity of Phosphoinositide 3-Kinase Inhibitor, Copanlisib in ABL Tyrosine Kinase Resistant Leukemia Cells
3128. Divergent Lineage-Specific Functions of PP2A in Chronic Phase CML and Lymphoid Blast Crisis
3129. Downregulation of BRCA1 Protein in BCR-ABL1–positive Cells Depends on Tiar-Mediated Repression of BRCA1 mRNA Translation
3130. Inhibition of CML Stem Cell Growth By Targeting WNT Signaling Using a Porcupine Inhibitor
3131. Glutathionylation of NF-Kb and Procaspase-3 Regulates Inducible Nitric Oxide Synthase Expression and Apoptosis of Chronic Myeloid Leukemia Cells
3132. Modeling Chronic Myeloid Leukemia in Immunodeficient Mice Reveals an Inflammatory State with Expansion of Aberrant Mast Cells and Accumulation of Pre B Cells
3133. Identification of New microRNA Biomarkers and Candidate Target Genes in Primitive CML Cells Using Global Comparative RNA analyses

10 Therapy: Poster II [3134-3160]

26 abstracts: https://ash.confex.com/ash/2014/webprogram/Session5970.html

3134. Increased Risk of Cardiovascular Events Associated with TKI Treatment in Chronic Phase Chronic Myeloid Leukemia. Data from Swedish Population-Based Registries
3135. Long-Term Follow-up of Ponatinib Efficacy and Safety in the Phase 2 PACE Trial
3136. Hyperhomocysteinemia and High Doses of Nilotinib Favour Cardio-Vascular Events in Chronic Phase Chronic Myelogenous Leukemia (CML) Patients
3137. Impact of Additional Cytogenetic Abnormalities and Variant t(9;22) at Diagnosis on Prognosis of Childhood Chronic Myelogenous Leukemia : The Experience of the International Registry for CML in Children and Adolescents *(I-CML-Ped Study)*
3138. A Two-Fold Rise of BCR-ABL Transcript Levels Advises BCR-ABL Mutation Analysis in Imatinib-Treated Chronic Myeloid Leukemia (CML) - an Analysis of the Randomized CML-Study IV
3139. A Profound Biological Difference of Chronic and Blast Phase Chronic Myeloid Leukemia in *Ex Vivo* Drug Responses
3140. Challenge to Use the Interval Censorship Estimators for Time to Response Evaluation By Data from Chronic Myeloid Leukemia Registry
3141. Five-Year Outcome of 215 Newly Diagnosed Chronic Myeloid Leukemia Patients Treated Frontline with Nilotinib-Based Regimens: A Gimema CML Working Party Analysis
3142. The Incidence of Pleural Effusion on Dasatinib Treatment Is Associated with CD56 Positive Cell Values One Month after Commencing Dasatinib and Achievement of an Early Molecular Response in Newly Diagnosed Chronic Myeloid Leukemia Patients: Results of a D-First Study
3143. Low Level BCR-ABL Mutations and Response to Treatment: A Substudy of the ENEST1st Trial
3144. Prognostic Value of Multi-Drug Resistance 1 Gene (MDR1) Expression in Newly Diagnosed Patients with Chronic Myeloid Leukemia on Nilotinib Treatment– a Subanalysis of the ENEST1st Study
3145. Incidence of CML in Europe - a Comparison of 19 European Countries with US SEER Data
3146. Long Term Outcomes of First Line Tyrosine Kinase Inhibitors for Chronic Phase Chronic Myeloid Leukemia: A Mixed-Treatment Comparison
3147. Incidence of Vascular Thrombotic Events in 183 Consecutive Patients Treated with Nilotinib: A Single Centre Experience
3148. Prognostic Value of the Rate of *BCR-ABL* Decline for Patients with Chronic Myelogenous Leukemia in Chronic Phase on Tyrosine Kinase Inhibitors Treatment
3149. Dasatinib Expands Pre-Existing, CMV-Associated, Highly Differentiated NK Cells in Ph+ Leukemia
3150. Integrating Molecular, Epigenetic and Pharmacogenetic Approaches in Managing Imatinib Resistance Among Malaysian Chronic Myeloid Leukemia Patients
3151. Cytogenetic and Molecular Responses in Patients with Chronic-Phase Chronic Myeloid Leukemia (CP-CML) in a Prospective Observational Study (SIMPLICITY)
3152. The Hasford Score Correlates with the Long-Term Molecular Response to Imatinib Treatment for Chronic Myeloid Leukemia Patients and May be Useful for Differentiating Low and Intermediate Risk Patients: A Single Institution Experience
3153. High-Resolution Analysis of the Relationship Between Dose and Molecular Response in CP-CML Patients Treated with Ponatinib or Imatinib
3154. Benefit-Risk of Ponatinib Vs. Bosutinib in Chronic Phase Chronic Myeloid Leukemia (CP-CML) Patients Who Failed Two Prior Tyrosine Kinase Inhibitors (TKIs): An Indirect Comparison
3155 Results from the Korean Imatinib Discontinuation Study (KIDS): Updated Data with 15-Month Median Follow up
3156. Frequency and Impact of Molecular Response's with Nilotinib (Tasigna) in Patients (Pts) with Newly Diagnosed Philadelphia Chromosome (Ph)-Positive Chronic Myelogenous Leukemia in Early Chronic Phase (CML-CP)
3157. Frequency of a Highly Polymorphic Variant, S36N *GFI1*, Is Increased in CML Patients
3158. Achieving Complete Cytogenetic Response and BCR-ABL PCRIS <1% within 12 Moths Are Important Goals for Imatinib Therapy in Newly Diagnosed, TKI-Naive Chronic Myeloid Leukemia Accelated Phase Patients
3159. Impact of Ph+ Stem Cell Burden on Clinical Findings and Molecular Responses to First-Line Nilotinib in Newly Diagnosed Chronic Myeloid Leukemia: The Results from the Interim Analysis of N-Road, Multi-Center a Phase II Study
3160. Baseline Characteristics of CML Patients Accross Europe - Comparing Real-World Patients with Patient Collectives Included in Clinical Trials

11 Biology and Pathophysiology, excluding Therapy: Poster III [4508-4530]

22 abstracts: https://ash.confex.com/ash/2014/webprogram/Session5969.html

4508. Effective and Selective Elimination of CML Stem Cells Using Novel Ethacrynic Acid Derivatives
4509. Contribution of Leukemia-Induced Alterations in Mesenchymal Cell Subpopulations to Altered Regulation of Leukemic and Normal Stem Cells in the CML BM Microenvironment
4510. Infrared Microspectroscopy Allows Direct Identification of Leukemic Cells Expressing T315I-Mutated BCR-ABL Via a Unique Spectral Signature
4511. Peptides from Nme2 Are Preferentially Expressed By Chronic Phase CML Cells, Recognized By T Cells and Restricted By the Most Frequent HLA-Antigens

4512. BGB324 Represents an Axl and BCR-ABL1 Inhibitor with Activity in the T315I Mutant
4513. Molecularly Defined Clonal Evolution in Patients with Chronic Myeloid Leukemia Independent of the *BCR-ABL* Status
4514. Activating Mutations Observed in De Novo Acute Myeloid Leukemia Are Also Present in a Subset of Philadelphia Chromosome (Ph)-Positive Leukemia Patients with BCR-ABL1-Independent Resistance to ABL1 Kinase Inhibitors
4515. Modeling Ponatinib Resistance in *BCR-ABL1*+ Cell Lines: Implications for Ponatinib Resistance in TKI-Resistant and TKI-naïve Patients
4516 CML Patients with Resistance to Tyrosine Kinase Inhibitors and without *BCR-ABL1* Resistance Mutation Frequently Carry Other Gene Mutations
4517. Chaetocin Anti-Leukemia Activity Against Chronic Myelogenous Leukemia Stem Cells Is Potentiated By Bone Marrow Stromal Factors and Overcomes Innate Imatinib Resistance
4518. Mapping the HLA Ligandome Landscape of Chronic Myeloid Leukemia (CML) - Towards Peptide Based Immunotherapy
4519. Synthetic Anti-IL3 Receptor Antibodies As Therapeutics to Block Innate Imatinib Resistance in Chronic Myelogenous Leukemia
4521. Increased p53 Acetylation By SIRT1 Inhibition Is Required for Optimal Activation of p53 Activity and Significantly Enhances the Ability of HDM2 Inhibitors to Target CML LSC
4522. Reduced Expression of CD62L Is Associated with Increased ADAM17 Activity and Predicts Molecular Response to Nilotinib Therapy in Patients with Early Chronic Phase Chronic Myelogenous Leukemia (CML-CP)
4523. Transphosphorylation of Endogenous BCR Mediates the Effect of T315I on the Transformation Potential of BCR/ABL
4524. Cellular Reprogramming Erases Aberrant DNA Methylation and the Malignant Phenotype in Chronic Myeloid Leukemia
4525. Persistence of Abnormally-Spliced, Functionally-Dead BCR-ABL Variants Is a Critical Obstacle to Achieve Sustained Complete Molecular Response in CML Patients: Results of a Quantitative, Highly-Sensitive, Deep Sequencing Study
4526. Next Generation Sequencing Identifies DNA Methylation Patterns Indicative of Disease Progression in Ph+ CML
4527. Regulation of Self-Renewal through Modulation of Let-7 Stem Cell Regulatory Family of microRNAs in Chronic Myeloid Leukemia Stem Cells
4528. Genetic Depletion of Fc Gamma Receptor 2b Affects CML Stem Cell Biology
4529. MiR-300 Acts As a Tumor Supressor in Ph+ Progenitors By Modulating the JAK2-SET/PP2A-Bcatenin Interplay
4530. GADD45a Is a Tumor Suppressor in BCR-ABL-Driven Leukemogenesis

12 Therapy: Poster III [4531-4568]

37 abstracts: https://ash.confex.com/ash/2014/webprogram/Session5971.html

4531. Ultra-Deep Sequencing Leads to Earlier and More Sensitive Detection of the TKI Resistance Mutation p.T315I in CML
4532. Deep Molecular Response to Nilotinib As First-Line Treatment of BCR-ABL+ CML in Early Chronic Phase: A Phase 3b Multicenter Study of the Gimema CML Working Party
4533. *MSI2* and *TGFβR1* Expression Levels in CML Patients and Hematopoietic Cell Lines
4534. The Observed and Expected Incidence of Cardiovascular (CV) Ischemic Events in Dasatinib (DAS)-Treated Patients (pts) Across a Clinical Trial Program
4535. Ponatinib As Frontline Therapy for Patients with Chronic Myeloid Leukemia in Chronic Phase (CML-CP)
4536. A Novel DNA Repair Chip Assay Allows Rapid Identification of DNA Repair Abnormalities Induced By BCR-ABL
4537. Management of Adverse Events Associated with Dasatinib during Early Periods of Therapy in the Treatment of Chronic Myeloid Leukemia -a Clinical Report of Daria-01 Study
4538. Correlation of Lymphocyte Count with Treatment Response to Tyrosine Kinase Inhibitors in Newly Diagnosed Chronic Myeloid Leukemia in Chronic Phase
4539. Dasatinib Plus Smoothened (SMO) Inhibitor BMS-833923 in Chronic Myeloid Leukemia (CML) with Resistance or Suboptimal Response to a Prior Tyrosine Kinase Inhibitor (TKI): Phase I Study CA180323
4540. Exceeding MR5.0 Sensitivity in Routine BCR-ABL1 Analysis Using Multiplex Digital PCR
4541. Efficacy and Safety of Nilotinib (NIL) vs Imatinib (IM) in Patients (pts) With Newly Diagnosed Chronic Myeloid Leukemia in Chronic Phase (CML-CP): Long-Term Follow-Up (f/u) of ENESTnd
4542. Efficacy and Safety of Dose-Optimized Nilotinib (NIL) in Patients (Pts) with Newly Diagnosed Chronic Myeloid Leukemia in Chronic Phase (CML-CP): ENESTxtnd Interim Analysis
4543. A Novel Approach to a Retrospective Longitudinal Analysis of Dose Change or Discontinuation of Imatinib Therapy in Chronic Phase - Chronic Myeloid Leukemia
4544. No Differences in Outcomes Between Patients Achieving Early Molecular Response at 3 Months and Those Achieving Optimal Response at 6 or 12 Months in Chronic Phase of Chronic Myeloid Leukemia Treated with Front-Line Imatinib: Taiwan CML Study
4546. Impact of Dose Intensity of Ponatinib on Selected Adverse Events: Multivariate Analyses from a Pooled Population of Clinical Trial Patients
4547. Attitudes and Perceptions of Patients (pts) with Chronic Myeloid Leukemia in Chronic Phase (CML-CP) Toward Treatment-Free Remission (TFR)
4548. Retrospective Analysis to Correlate Impact of Symptom Burden and Quality of Life to Treatment Outcome with Tyrosine Kinase Inhibitors in Chronic Myeloid Leukemia Chronic Phase
4549. Effectiveness in Predicting the Response and Outcome with Three Prognostic Scoring Systems in Pediatric CML CP on Upfront Imatinib
4550. Evaluation of Comorbidities Relevant to Tyrosine Kinase Inhibitor Treatment Among Patients with Chronic Myelogenous Leukemia in the U.S. Managed Care Setting
4551. Comparative Efficacy Among 3rd Line Post-Imatinib Chronic Phase-Chronic Myeloid Leukemia (CP-CML) Patients after Failure of Dasatinib or Nilotinib Tyrosine Kinase Inhibitors
4552. Ponatinib Efficacy and Safety in Patients with the T315I Mutation: Long-Term Follow-up of Phase 1 and Phase 2 (PACE) Trials
4553. Long-Term Follow-up Results after Imatinib Discontinuation in Chronic Myeloid Leukemia Patients with Undetectable Minimal Residual Disease
4554. Retrospective Analysis of Allogeneic Transplant Outcomes in Chronic Myeloid Leukemia Patients with Tyrosine Kinase Inhibitors Failure
4555. Comprehensive Quantitative RT-PCR Detection of Multiple *BCR-ABL1* mRNA Species for Diagnosis and Monitoring of Leukemias
4556. Proteomics–Based Approach Predicts Molecular Response and Stratifies Responders to Tyrosine Kinase Inhibitors (TKI) in Chronic Myeloid Leukemia (CML) Patients
4557. Clinical Outcome Following Change of Tyrosine Kinase Inhibitor (TKI) According to the Detection of an ABL Kinase Mutation
4558. Long-Term Follow-up of a Phase 1 Study of Ponatinib in Patients with Chronic-Phase Chronic Myeloid Leukemia (CP-CML)

4559. Bosutinib As Third-Line Therapy in Patients (Pts) with Chronic Phase Chronic Myeloid Leukemia (CP CML) Following Failure with Imatinib Plus Dasatinib and/or Nilotinib: 48-Month Update of a Phase 1/2 Study
4560. Index to Predict MMR at 12 Months in Chronic Phase Chronic Myeloid Leukemia Patients Based on the Area Under the Lymphocyte Curve
4561. Achieving the Deep Molecular Response Levels Required for an Imatinib Discontinuation Trial Is Strongly Associated with the BCR-ABL Level at the First Qualifying Timepoint
4562. Model-Based Characterization of the Molecular Response Dynamics of Tyrosine Kinase Inhibitor (TKI)-Treated CML Patients – a Comparison of Imatinib and Dasatinib First-Line Therapy
4563. The Role of the Newer Tyrosine Kinase Inhibitors As First Line Treatment in Chronic Phase Chronic Myeloid Leukemia – an Updated Meta-Analysis
4564. BCR-ABL1 Transcript of 7.93% at 3 Months Is an Early Predictor for Long-Term Survival to Second-Line Therapy Using Next Generation Tyrosine Kinase Inhibitors in Imatinib-Resistant Chronic Phase Chronic Myeloid Leukemia
4565. Phase II Clinical Trial Results of Dasatinib for Frontline Therapy in Patients with Chronic Myeloid Leukemia (CML) in Chronic Phase (CP)
4566. Paper or Plastic: RT-PCR of BCR-ABL from Blood Spots Stored and Shipped on Paper
4567. Population Based Study of Cytogenetic Abnormalities in Addition to Philadelphia (Ph) Chromosome in Patients with Chronic Myeloid Leukaemia (CML) and Impact on Survival
4568. Imatinib Combined with Reduced-Intensity Allogeneic Hematopoietic Stem Cell Transplantation Versus Imatinib Alone for Young Patients with Chronic Myeloid Leukemia in Chronic Phase