Abstracts and notes on CML presentations
ASH 2008 San Francisco

Steve O'Brien

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1 Highlights

I have not aimed to review all the abstracts, rather pick up the main themes. I have focused mainly on the oral presentations and clinically relevant studies. Complete abstracts are included for all oral presentations and for many of them I've added notes from the presentations. I've taken care to ensure the accuracy of the data but when rapidly typing during sessions I can't always guarantee complete precision!

Abstracts are available on line at: http://ash.confex.com/ash/2008/webprogram/start.html

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

- First release of the data from SPIRIT studies and additional data evaluating imatinib dose (see section 1.2). Does IFN in combination have a role? Is more imatinib better?
- 2nd generation drugs (nilotinib and dasatinib) in first line chronic phase (see section 1.6). How good are they? How do they compare?
- Whether or not you can safely stop imatinib (see section 1.5). 2 studies offer early insights.

1.1. Imatinib update

- **7 yr update on IRIS** [186] (see section 2.6)**.** Data still continue to look pretty good. 82% achieved CCR and KM overall survival is 86%. Of 456 (out of 553) achieving CCR, 79 (17%) lost response over time. At 7 years 60% patients were still on 'study' imatinib – obviously 40% had come off study drug over that period of time so there is perhaps room for improvement with newer TKIs.
- Is CCR a 'safe haven'? Of the 456 patients who achieved a CCR15 (3%) developed AP or BC and 10 patients died from CML. However in patients who maintained their CCR for more than three years, the progression rate was zero. Achieving CCR can take some time: 33 patients (7%) took more than two years to achieve CCR and their outcome was just as good as early responders.
- **IRIS PCR data.** [334] (see section 4.4). Patients with greater than 1% BCR-ABL/ABL ratio at 12 months seem to do less well in terms of EFS and progression to AP/BC. As with the cytogenetic data, achieving a late MMR is just as good as achieving an early response in terms of freedom from progression. Achieving a MMR seems to be a 'safe haven', even more so than CCR.
- **Plasma levels**: data from TOPS (400 vs 800) [447] (see section 5.3). Response is related to plasma level. E.g. MMR is siginificantly less common at trough levels below 1165 ng/ml. Correspondingly grade ¾ neutropenia was much more frequent in the quartile above 3180 ng/ml. It's still uncertain whether plasma monitoring should be adopted in regular clinical practice.

- **Transplant after imatinib.** Not much presented but data from German CML IV study [448] (see section 5.4) indicated that outcome of BMT following imatinib may be 'better than expected'.

1.2. Imatinib dose studies: SPIRIT France, Germany and others

- **Data from the SPIRIT studies**. The French SPIRIT study [183] (see section 2.3) and the German SPIRIT study [184] (see section 2.4) were both presented for the first time. Early days but it seems from the French as though the IFN-containing arm produces better molecular response at 12 months. It is too early to evaluate impact on survival. The German study did not however confirm this observation and the Germans have also chosen not to unblind their study so far. It remains uncertain whether the addition of IFN to imatinb confers an advantage. The UK SPIRIT study continues to address this question and we will hopefully submit data for ASH in 2009.

- **Is more imatinib better?** Probably not… The GIMEMA/ELN study [185] (see section 2.5) and the TOPS study [335] (see section 4.5) both compared 400 vs 800mg daily in newly-diagnosed CML patients. Although speed of responses seem to be quicker at the higher does, no significant difference was seen in either study in one year molecular or cytogenetic response. It is too early to detect any differences in survival but it seems unlikely that such differences will emerge given the similarity of one-year response. 400mg imatinib should probably remain the baseline comparator for newer studies of TKIs.

1.3. Predicting response to imatinib

- Early molecular response to first line imatinib may predict EFS [333] (see section 4.3). Similar story with IRIS [334] (see section 4.4). Novel biomarkers may also predict response [336] (see section 4.6).

- A study from the MD Anderson evaluated the significance of rising levels of BCR-ABL in patients with CCR [445] (see section 5.1). In 116 patients with a documented CCR who then had an increasing level of PCR. 9% of patients with increasing PCR suffered subsequent disease progression. It was emphasized that there are no data to suggest that early intervention in this situation confers any survival benefit.

1.4. Imatinib resistance & mutations

- A useful epidemiological study from 9 countries [188] (see section 2.8) has confirmed the poor outcome of patients with the T315I mutation. 222 patients – probably one of the largest series.

- Data were presented on 1150 patients treated with 2nd line dasatinib from the START studies [449] (see section 5.5). A really impressive piece of work. 402 (39%) patients had baseline mutations: 64 mutations affecting 49 amino acids. Interestingly there was no difference in all response criteria between patients with and without mutations. No difference in survival was observed.

1.5. Imatinib – time to stop?

- There's no definitive answer to this question as yet but useful data are accumulating. Two groups presented their results: the French group [187] (see section 2.7) and the Australian group [1102] (see section 8.2).

- In an initial French pilot study, the longest that anyone has gone without imatinib and remained in remission is 4 years. In the French 'STIM' study patients had to be in CMR for at least 2 years with imatinib. 69 patients were included. 60 patients had more than 1 month follow up. 31 of 60 had previously received IFN and 29 of 60 were *de novo* patients. The median follow up was 5 months. Of 60 patients analysed, 28 patients had relaplsed molecularly. In those who did relapse molecularly, all patients remained sensitive to imatinib rechallenge.

- Data from the Australian study were not as mature [1102] (see section 8.2) but still encouraging. The Adeleide group assessed 18 patients, 13 of whom had previously received IFN. 10 of 13 prior IFN patients (7 for at least 12 months) and 3 of 5 *de novo* patients remain in CMR. Any patients who relapsed remained responsive to imatinib.

- There's a suggestion that staying in remission after stopping therapy may correlate with previous exposure to IFN but this is far from certain from the current data.

1.6. First line use of second generation TKIs: nilotinib and dasatinib.

The table below is *broad comparison* of the **newly-diagnosed (first line) chronic phase data**. *These data are not strictly comparable* as they are from different studies with different follow up periods and slightly different entry criteria and patient groups. Of note:

- The follow up time is just under a year less for nilotinib than dasatinib.
- Comparing like for like, there is probably not much difference in efficacy or durability of responses so far.
- Cytopenias seem perhaps more common with dasatinib.
- Phase 3 studies comparing these new agents with imatinib are ongoing including the SPIRIT2 trial in the UK.

	Nilotinib 400 bid	Nilotinib 400 bid	Dasatinib 100, 50[2]
Centre	MD Anderson	GIMEMA	MD Anderson
Abstract	[446] (see section 5.2)	[181] (see section 2.1)	[182] (see section 2.2).
No. patients total	53 (CP)	73	52
Median age		51	
Median follow up (range)	12 months	337 days	Longest FU is 30/12
Median dose delivered		75% achieved 800mg	
CHR	100		
CCyR	98%	96% at 6/12	98%
MMR	40% at 12 months	66% at 6/12	50%
¾ neutropenia	11%	4%	
¾ thrombopenia	9%	3%	

1.7. Second line dasatinib and nilotinib

- There was not much emphasis on second line TKI data this year. With both drugs CCR rates seem to be of the order of 50% and there is not much to choose between them in terms of toxicity. Unfortunately no studies directly comparing these two agents are being conducted. This author thinks there should be.

- Data on time to response and durability of response in dasatinib-treated patients [450] (see section 5.6) were presented.

- A study from Australia involving 155 patient (73 nilotinib, 82 dasatinib) looked at the value of molecular response at 3 months in predicting major molecular response at 24 months in patients receiving 2nd generation TKIs [331] (see section 4.1).

- A UK study sought to evaluate predictive factors for response to 2nd generation TKI [332] (see section 4.2). 80 patients CP-CML patients (dasat 67, nilot 13) were evaluated. Median follow up was 28 months. A number of factors were predictive of good response; best cytogenetic response on IM, Sokal score and neutropenia during IM therapy seemed to be most important. A scoring system has been devised. Failure to achieve a MCR at 6 months is associated with worse prognosis.

1.8. Other drugs: in early-stage clinical trials

- **Bosutinib (SKI606).** There was an update on patients receiving bosutinib second line [1098] (see section 8.1). Data on 283 patients were reported. Of 84 patients evaluable for cytogenetic response, CCR rates were between 29% (imatinib-resistant) and 50% (imatinib intolerant). Not so different from nilotinib and dasatinib given the variable follow up times. The drug looks promising in terms of response rates and appears to be well tolerated. A study evaluating bosutinib as first line is currently being conducted – no data so far. Rash and diarrhoea occasionally problematic – 7% grade ¾ tox for both. Low rates of cytopenia, mild nausea common.

- Data on **INNO406**, a compound structurally quite similar to nilotinib, were presented at ASH in 2007. The study opened about 2 years ago and 49 patients had been enrolled. I could not find a clinical update on this compound at ASH 2008.

- **PHA739358** is active against T315I [abstract 1030 at *ASH 2007*]. Seven patients were presented last year and 2 responses in patients with T315I had been observed. Very early and again it's an IV drug. I could not find a clinical update on this agent at ASH 2008 – not sure why.

- **MK0457**, another compound acive against T315I, has been dropped from further development by Merck. It was quite difficult to administer (5 day IV infusion) and had significant toxicity. Uncertain whether it will now ever see the light of day in the clinic. There didn't seem to be any abstracts on this compound at the meeting this year.

- **HHT** continues to be evaluated. Although an 'old style' chemotherapy drug rather than say a TKI, results are reasonable and it may have utility in T315I. There was not much interest in the drug this year.

1.9. Other drugs: look promising, not yet in clinical trials

- **XL228** is one of the new kids on the block and the first clinical data were presented in poster form [3232] (see section 8.3). Produced by Exelixis (San Francisco), XL228 is a potent multi-targeted protein kinase inhibitor with activity against IGF1R, src, and Abl. It displays low nanomolar biochemical activity against wild type Abl kinase (K_i = 5 nM), *as well as the T315I* form of Abl resistant to imatinib and dasatinib (Ki = 1.4 nM). Early clinical results were presented on just **xx** patients. The drug is given as a once weekly IV infusion over an hour and so far the study is only at the stage of dose finding. There is, as yet, no pharmacodynamic evidence of target inhibition *in vivo*.

- **DCC2036** [576] (see section 6.8), a so-called 'switch pocket' inhibitor made by Deciphera (Lawrence, Kansas), has activity against T315I and is effective in mice transfected with T315I leukaemia cells [463]. Clinical trials are due to start in 2009. A similar compound called DCC2157 [576] (see section 6.8) is also being evaluated.

- **FTY720**. PP2A inactivation may kill CML cells. Interesting provisional data were presented on a compound called FTY720 - published in Cancer Cell in November 2008 - Neviane *et al.* [189] (see section 3.1). This compound offers a novel mechanism of action which is not TK-dependent.

- There was an update on **AP24534** [726] (see section 7.6) which again looks fairly promising. AP24534 is an oral, multi-targeted kinase inhibitor with activity against native and kinase domain-mutant BCR-ABL, including T315I. A phase 1 clinical trial designed to evaluate AP24534 treatment in patients with refractory CML and other hematologic malignancies has recently commenced.

2 Chronic myeloid leukemia - clinical trials 1 [abstracts 181 – 188]

2.1. [181] High and Early Rates of Cytogenetic and Molecular Response with Nilotinib 800 Mg Daily as First Line Treatment of Ph-Positive Chronic Myeloid Leukemia in Chronic Phase: Results of a Phase 2 Trial of the GIMEMA CML Working Party

Rosti *et al*, Italy. Imatinib (IM) 400 mg daily is the standard treatment for chronic myeloid leukemia in early chronic phase (ECP): the results of the IRIS trial have shown a 72 months overall survival of 95%; EFS and PFS were 83% and 93%, respectively; the

cumulative rate of complete cytogenetic response (CCgR) for the IM 400 mg arm was 25% at 3 months (at 6, 12, 18 and 60 months it was 51%, 69%, 76% and 87%, respectively). Nilotinib, a second generation TKI, has a higher binding affinity and selectivity for Abl with respect to IM, being 20 to 50 times more active in IM-sensitive cell lines and is highly effective in IM resistant patients, across every disease phase. To investigate the therapeutic efficacy and the safety of nilotinib 400 mg BID in untreated, ECP, Ph-pos CML patients, the italian GIMEMA CML Working Party is conducting an open-label, single stage, multicentric, phase II study trial (ClinicalTrials.gov. NCT00481052); all patients provided written informed consent. The primary endpoint is the CCgR rate at 1 year; the kinetic of molecular response is studied by Q-PCR baseline and after 1, 2, 3, 6, 9 and 12 months from treatment start. PATIENTS Seventy-three patients have been enrolled from 20 Centres between June, 2007 and February, 2008. The median age was 51 years (range 18-83), 45% low, 41% intermediate and 14% high Sokal risk. Median follow-up is currently 210 days (range 68-362). RESULTS All 73 patients and 48/73 (66%) completed 3 and 6 months on treatment, respectively. Response at 3 and 6 months (ITT): the CHR rate was 100% and 98%, the CCgR rate 78% and 96%, respectively. A MMR, defined as a BCR-ABL:ABL ratio < 0.1% according to the International Scale, was achieved by 3% of all treated patients after 1 month on treatment, but this proportion rapidly increased to 22% after 2 months, 59% after 3 months and 74% after 6 months. One patient progressed at 6 months to accelerated-blastic phase with the T315I mutation. NILOTINIB DOSE AND COMPLIANCE No dose escalation was permitted in case of resistance; the median daily average dose was close to the intended dose, 789 mg (range 261 – 800); 34/73 patients (47%) interrupted nilotinib at least once, with a median duration of dose interruption of 15 days (range 2-98). The dose of nilotinib at the last visit was 400 mg BID for 52 patients (71%), 400 mg daily for 20 patients (27%) and 200 mg daily for 1 patient (1%). ADVERSE EVENTS: AEs (grade III/IV) were manageable with appropriate dose adaptations: hematologic toxicity was recorded so far in 4 pts (5% - only 1 event grade IV neutropenia); the most frequent biochemical laboratory abnormalities (grade III) were total bilirubin increase (15%), GOT/GPT increase (11%) and lipase increase (4%). Only 1 episode of grade IV lipase increase was recorded. It is noteworthy, considering the 48 cases with at least 6 months of follow-up, that the incidence of any grade II and III non-hematologic adverse event, decreased from 50% and 8% (first 3 months) to 23% and 6% (second trimester), respectively. ECG monitoring: in 16 patients (22%), transient and not clinically relevant ECG abnormalities have been recorded; 2 more patients (3%) revealed a transient and uneventful QTc prolongation (>450 but <499 msec). CONCLUSIONS: The results that have been achieved in these unselected patients and within a multicentric trial, strongly support the notion that in ECP Ph-pos CML patients both cytogenetic and molecular responses to nilotinib are substantially faster than the responses to IM.

NOTES ON PRESENTATION

Protocol CML 0307. Phase 2 study. 18 centres. 400mg bid nilotinib during a fasting period. No dose escalation. Primary endpoint CCR at one year. June 06 to Jan 07. 54/41/14 Sokal H to L. 2 dropped out – 1 for AP/BC and one due to intolerance.

<template>	**Overall**		**Comment**
Parameter	No.	%	
No. patients total	73	100	
No. patients analysed			
Median age	51		
Prior mutations			
Median previous disease duration			
Median follow up (range)	337		
Median dose delivered	800mg in 75% at 6 months. 400mg 25%		Older patients tolerate dose just as well.
CHR			
MCyR			
CCyR		96% at 6 months	Older patients achieved the same level of response as younger patients.
MMR		66% at 6 months	
Median survival			
¾ anaemia			
¾ neutropenia		4	

¾ thrombopenia		3	
Lipase		4 gd 3/4	
ALT			
Pleural effusion ¾			
QTc prolongation		3	
Rash ¾		42 all/5 gd. 3,4	
Diarrhoea ¾			
Bilirubin		16 gd ¾	

2.2. **[182] Efficacy of Dasatinib in Patients (pts) with Previously Untreated Chronic Myelogenous Leukemia (CML) in Early Chronic Phase (CML-CP).** Cortes *et al*, Houston. Background: Dasatinib (BMS-354825) is a multi-targeted kinase inhibitor of BCR-ABL and SRC with significant activity in pts with CML-CP resistant to or intolerant of imatinib (IM). We initiated a phase II trial to study efficacy and safety of dasatinib in pts with previously untreated CML-CP. Aims: To investigate the efficacy and safety of dasatinib as initial therapy for patients with CML-CP. Methods: The primary objective was to estimate the proportion of pts attaining major molecular response (MMR) at 12 months (mo). Pts with previously untreated CML-CP were eligible and received dasatinib 100 mg/day, randomized to either 50 mg-twice-daily (BID) or a 100 mg-once-daily (QD). Results: Fifty pts have been enrolled (25 on the QD schedule, 25 BID). Median age was 45 years (yrs) (range 18–76 yrs); 75% are Sokal low risk. Median follow-up is 24 months (mo). Overall, 44/45 (98%) evaluable patients achieved complete cytogenetic response [CCyR]. The CCyR rate at 3, 6 and 12 mo compares favorably to that observed in ***historical*** controls treated with imatinib 400mg or 800 mg daily:

Mo on therapy	Percent with CCyR (No. evaluable)			P value
	Dasatinib	Imatinib 400mg	Imatinib 800mg	
3	78 (45)	37 (49)	62 (202)	0.0003
6	93 (41)	54 (48)	82 (199)	<0.0001
12	97 (35)	65 (48)	86 (197)	0.0001
18	88 (33)	68 (38)	89 (179)	0.004
24	80 (25)	70 (40)	88 (173)	0.006

MMR was achieved in 12/35 (34%) at 12 mo and 12/25 (48%) at 18 mo. One of 46 (2%) evaluable pts have achieved confirmed complete molecular response, and 1 other unconfirmed (ie, only achieved on their last assessment). Grade 3-4 non-hematologic toxicity (regardless of causality) included pruritus (13%), fatigue (6%), neuropathy (4%), and memory impairment (4%). Pleural effusion occurred in 21% evaluable pts (grade 3-4 in 2%). Grade 3-4 hematologic toxicity (transient) was thrombocytopenia in 11%, neutropenia in 21%, and anemia in 9%. Twenty-seven (54%) pts required transient treatment interruption. The actual median daily dose for all pts was 100mg. There is no significant difference in grade 3-4 toxicity by treatment schedule. Four pts came off study: 1 pts choice after 1 dose, 1 for toxicity (pleural effusion, QD schedule), and 2 lost response after multiple treatment interruptions (1 myelosuppression, 1 pleural effusion, both BID schedule). Two other pts have lost response because of non-compliance. 24 month EFS (event = loss of CHR, loss of MCyR, AP/BP, death, or off because of toxicity) is 81%. Conclusion: Rapid CCyR occurs in most patients with previously untreated CML-CP treated with dasatinib frontline therapy with a favorable toxicity profile. Accrual to this trial continues

NOTES ON PRESENTATION

Primary endpoint is MMR. <1month of prior imatinib or IFN (?flaw). Randomise 50x2 vs 100x1. 5 patients off study.

<template>	**Overall**	**100od**	**50bd**	**Comment**

Parameter	No.	%	No.	%	No.	%	
No. patients total	52						
No. patients analysed	49						
Median age							
Prior mutations							
Median previous disease duration							
Median follow up (range)	Longest is 30/12						
Median dose delivered							
CHR	49	100					
MCyR							
CCyR	47/48	98					
MMR	24/48	50		29		? lower	
Median survival		84% EFS at 24/12.					All patients alive
¾ anaemia							
¾ neutropenia			23				
¾ thrombopenia			12				
Lipase							
ALT							
Pleural effusion ¾	1 grade 3						10 patients. 2 had to stop because of pleural effusions
QTc prolongation							
Rash ¾							
Diarrhoea ¾							
Treatment interruptions		54					

2.3. **[183] Randomized Comparison of Imatinib Versus Imatinib Combination Therapies in Newly Diagnosed Chronic Myeloid Leukaemia (CML) Patients in Chronic Phase (CP): First Results of the Phase III (SPIRIT) Trial from the French CML Group (FI LMC).** Guilhot *et al.* Imatinib (IM) at 400 mg daily is the first line therapy for newly diagnosed CML patients (pts); however, less than 50% of major molecular responses (MMR) are obtained at 12 months. To improve these results, we designed a phase III, multicenter, open-label, prospective randomized trial. The reference arm was IM 400 mg daily (n=159). The 3 experimental arms were IM 600 mg daily (n=160), IM 400mg daily in combination with Ara-C, (20 mg/m2/day, days 15-28 of 28-day cycles)(n=158) and IM 400mg in combination with Peg-IFN alfa-2a (Peg-IFN2a, 90 µg weekly) (n=159). Treatment was delivered at least 12 months or until treatment failure (disease progression) or major toxicity. The primary endpoint is the overall survival. Other endpoints are: rate and duration of hematologic and cytogenetic responses, major (MCyR) and complete (CCyR), molecular response (major molecular response ie MMR) and the tolerability. Using treatment allocation ratio 1.1.1.1, randomization was stratified according to Sokal risk groups. The current interim analysis of the first 636 patients (α=0.85%, β=10%) at 1 year from randomization was planned in order to select the best experimental arm for further comparison with IM 400. The increased dose of IM or a combination regimen would be considered as promising if it increased the 4 log reduction response rate by at least 20 percentage points, e.g. from 15% to 35%, with an acceptable tolerability. Evaluation of molecular response up to 12 months was centralized, blinded and calculated according to International score (IS). Pts were recruited between 9/2003 and 10/2007.[median age 51 yrs (18-82), 62% of pts were male; Sokal distribution was low risk 33%, intermediate risk 41% and 27% high risk]. Median follow-up is 36 months (range 8-57) at the time of analysis. Overall, at 3 months 86 % of pts achieved complete hematologic response. The MCyR, CCyR and MMR rates at 6 and 12 months are:

	IM-400	IM-600	IM-Ara-c	IM-PegIFN

At 6 months (636 pts, ITT) MCyR CCyR *	 74% 48%	 79% 67%	 68% 55%	 74% 56%
At 12months (562 evaluable pts) MCyR CCyR	 64% 57%	 76% 65%	 77% 66%	 74% 71%
MMR at 6 months**	21%	33%	27%	39%
MMR at 12 months	40%	52%	51%	61%

*p< 10-2 (overall); ** p<10-2 (overall)

Interestingly the rate of MMR at 6 months was significantly higher for IM-PegIFN as compared with IM-400 (p<10-3). The 4-log reduction rate in the BCR-ABL/ABL transcript were 18%, 21%, 22%, 34%, for the IM-400, IM-600, IM-Ara-c and IM-PegIFN arms respectively. The corresponding numbers of undetectable (complete molecular response) pts were 2%, 2%, 3% and 9% at 12 months respectively. Grade 3/4 neutropenia and/or thrombocytopenia occurred in 8% of IM-400 pts, in 14% of IM-600 pts, in 41% of IM-Ara-c pts and in 40% of IM-PegIFN pts respectively. Grade 3/4 non hematological events were reported in 19% of IM-400 pts, in 30% of IM-600 pts, in 27% of IM Ara-c pts and in 31% of IM-PegIFN pts. Among them a relationship between treatment and event was suspected for 21 pts (13%) with IM-400 (7 liver toxicity; 7 oedema+muscle cramps), for 31 pts (19%) with IM-600 (7 liver toxicity, 11 oedema+ muscle cramps) for 36 pts (23%) with IM-Ara-c (2 liver toxicity; 10 gut side effect) and for 47 pts (29%) with IM-PegIFN (6 liver toxicity, 13 skin rash). Discontinuation of experimental treatment occurred within the first 6 and 12 months in 26% and 18% of IM-Ara-c pts and in 35% and 11% pts of IM-PegIFN pts respectively. Within the first 12 months 36% of 600-IM pts reduced their dosage. Although a substantial number of pts stopped PegIFN, this first analysis indicates the usefulness of a combination of IM-PegIFN for the initial treatment of pts with CML CP with a significant molecular response rate improvement. Complete analysis of the 636 pts with a follow-up of 12 months will be presented.

NOTES ON PRESENTATION

Major endpoint overall survival. Interim analysis. Addition of PEG improves MMR rate. Significantly higher rates of neutropenia with IFN.

<template>	**1. Imat 400**		**2. Imat 600**		**3. Imat / IFN**		**4. Imat / Ara-C**		
Parameter	No.	%	No.			%	No.	%	
No. patients total	159		160			158		159	
No. patients analysed									
Median age									
Prior mutations									
Median previous disease duration									
Median follow up (range)									
Median dose delivered									
CHR									
MCyR									
CCyR		49		68			56		56
MMR		38		49		57		?	
Median survival									

¾ anaemia									
¾ neutropenia									
¾ thrombopenia									
Lipase									
ALT									
Pleural effusion ¾									
QTc prolongation									
Rash ¾									
Diarrhoea ¾									

Optimum molec. 0.01 peg 30 15 - 400, 18 - 600, 15 – Ara-C

2.4. **[184] Randomized Comparison of Imatinib 400 Mg Vs. Imatinib + IFN Vs. Imatinib + AraC Vs. Imatinib after IFN Vs. Imatinib 800 Mg: Optimized Treatment and Survival. Designed First Interim Analysis of the German CML Study IV.** Hehlmann *et al*. In spite of favorable response and survival results for the majority of CML patients on imatinib therapy, in a substantial minority imatinib fails or shows suboptimal responses. A treatment optimization study was therefore designed to compare in a randomized fashion standard imatinib vs. imatinib + interferon alpha (IFN) vs. imatinib + low dose araC vs. imatinib after IFN (for low- and intermediate-risk patients) or vs. imatinib 800 mg (for high-risk patients). Inclusion criteria were newly diagnosed BCR/ABL positive CML in chronic phase. In July 2005, randomization to the arms imatinib + araC and imatinib after IFN was discontinued and recruitment for imatinib 800 mg was expanded to low- and intermediate-risk patients. Primary goals are: rates of hematologic, cytogenetic and molecular remissions, duration of chronic phase, overall survival, adverse events and analysis of subsequent allografting. Since its activation in 7/2002, 1203 patients have been randomized. The current evaluation represents the first of three designed, statistically adjusted interim analyses of 710 patients randomized by the end of 2005 with a follow-up of at least 2 years. Analysis was according to intention to treat. 666 patients (545 with primary imatinib, 121 with primary IFN) were evaluable for hematologic, 621 for cytogenetic, and 631 for molecular responses. Median age was 53 years, 60% were male, median values were for Hb 12.5 g/dl, WBC 71.2/nl and platelets 384/nl, 35% had low, 53% intermediate and 12% high risk (Euro score). Median observation time was 3.5 years. Median duration of IFN pretreatment was <4 months. At 1 year, the cumulative incidence of complete hematologic remission (CHR) was 82.3% and 74.4%, of major cytogenetic remission (MCR) 65.6% and 40.6%, of complete cytogenetic remission (CCR) 52% and 19.7%, and of major molecular remission (MMR) 33.2% and 4.7% for primary imatinib and IFN therapies, respectively. At 3 years, the cumulative incidence of CHR was 96.4% and 93.8%, of MCR 89.5% and 89.1%, of CCR 85.2% and 78.5%, and of MMR 79% and 63% for primary imatinib and IFN therapies, respectively. 5-year-survival probability of all patients currently exceeds 90% (94% for imatinib-, 91% for IFN-based therapy, Figure 1). Event free survival after two years (no progression, no death, CCR within the first 18 months, no loss of CHR or MCR) was 80.3%. 36 patients died, 51 patients were transplanted in first chronic phase, and 80 patients progressed, 43 of which were switched to alternative treatments (16 to new drugs, 18 to transplantation, 9 received both). Type and severity of adverse events (AE) did not significantly differ from those reported previously. Hematologic AEs (leukopenia, thrombocytopenia) were most frequent in the imatinib 800 mg arm. Nonhematologic AEs (gastrointestinal) were most frequent in the combination arms and with imatinib 800 mg. In no case recruitment had to be changed due to superiority or inferiority of any arm. This applies also to the high dose imatinib arm where earlier response might translate into better survival. In conclusion, this first interim analysis shows favorable survival and long term response rates. Imatinib in combination with, or after, IFN or with low dose araC are feasible and equally safe treatment alternatives. More definite information will be provided by the next interim evaluation after recruitment has been terminated.

NOTES ON PRESENTATION

From July 2002. 715 patients presented. In study 710. Evaluable 694. Overall CCR at 5 years is 94%. Therapy arms NOT YET UNBLINDED. Imat after therapy D is lagging. OS at 6 years is 92%. CCR and MMR are 94 and 88.

The Germans did NOT see the same benefit as the French – came up in questions.

2.5. **[185] Cytogenetic and Molecular Response to Imatinib in High Risk (Sokal) Chronic Myeloid Leukemia (CML): Results of An European Leukemianet Prospective Study Comparing 400 Mg and**

800 Mg Front-Line. Baccarani *et al.* Sokal risk formulation was elaborated 25 years ago, based on very simple factors (age, spleen size, platelet count, and percentage of myeloblasts in the peripheral blood), based on patients treated with conventional chemotherapy. In spite of that, Sokal risk score is still the major prognostic factor for response to treatment with the tyrosine kinase inhibitor, Imatinib mesylate (IM). Since several preclinical, pharmacokinetic and clinical studies suggested that the therapeutic efficacy of IM may be concentration/dose–dependent, we assigned prospectively 217 adult patients with Ph pos CML, Sokal high risk (SHR), to be treated front line with IM 400 mg or 800 mg (Clin.Trials Gov. NCT00514488), comparing the cytogenetic and the molecular response rates at 3, 6, and 12 months. Cytogenetic response was evaluated by chromosome banding analysis (CBA) of marrow metaphases, and by FISH analysis of marrow cells in case of insufficient metaphase number. Molecular response was evaluated by RT-Q-PCR (PB), according to the international scale. The results are shown in Table 1. No difference between the two arms was significant at any time point. In the 400 mg arm, the median daily dose of IM was 400 mg, with 87% of patients receiving 350 to 400 mg. In the 800 mg arm, the median daily dose of IM was 720 mg, with 63% of patients receiving 600 to 800 mg. The CCgR rate was 86%, vs 66% in the patients who received a median daily dose of 600 to 800 or less than 600 mg daily, respectively (p=0.013). With a median follow up of 31 months (range 1-49 months), progression-free and overall survival are higher than 90% in both arms. Based on an intention-to-treat analysis, this study did not show a significant benefit of 800 mg over 400 mg in SHR patients, but the patients who could comply with the high dose had a better cytogenetic outcome.

NOTES ON PRESENTATION

Primary enpoint is CCR at 1 year. CCR and MMR are not different between different arms. 84-91% projected overall survival.

<template>	**Overall**		**Imatinb 400**		**Imatinib 800**		**Comment**
Parameter	No.	%	No.	%	No.	%	
No. patients total	216		108		108		
No. patients analysed							
Median age							
Prior mutations							
Median previous disease duration							
Median follow up (range)	26/12						
Median dose delivered					720		Only 50% of patients could tolerate full 800 mg. @800 CCR is 91%. @400-700 was 69.
CHR							
MCyR							
CCyR				58@1 12/12		64@1 2/12	NS
MMR				33		40	NS
Overall survival							NS
¾ anaemia							
¾ neutropenia							
¾ thrombopenia							
Lipase							
ALT							
Pleural effusion ¾							
QTc prolongation							
Rash ¾							
Diarrhoea ¾							
Withdrawls				27		31	

2.6. **[186] International Randomized Study of Interferon Versus STI571 (IRIS) 7-Year Follow-up: Sustained Survival, Low Rate of Transformation and Increased Rate of Major Molecular Response (MMR) in Patients (pts) with Newly Diagnosed Chronic Myeloid Leukemia in Chronic Phase (CML-CP) Treated with Imatinib (IM).** O'Brien *et al*. Background: Based on results from the IRIS trial, IM is the standard of care for pts with newly diagnosed CML-CP. This report presents the 7 yr data update of IRIS

to assess long term outcome, response rate, and safety in pts on primary IM therapy. Methods: 553 pts were randomly assigned to IM and evaluated for hematologic, cytogenetic and molecular responses, discontinuations/cross-over reasons, event-free survival (EFS), progression to accelerated-phase (AP) or blast crisis (BC) and OS. Events for EFS were defined as the first occurrence of any of the following during treatment: death from any cause, progression to AP/BC, loss of a complete hematologic response or major cytogenetic response (MCyR), or an increasing white blood cell count to > 20 x 109/L. After discontinuation of study treatment, pts were followed only for OS. Results: At 7 yrs, the estimated EFS was 81%, freedom from progression (FFP) to AP/BC was 93%, and the estimated OS was 86%. The best observed rates for MCyR and complete cytogenetic response (CCyR) were 89% and 82%, respectively. A total of 317 (57%) of all randomized pts remained on IM per protocol and were in CCyR. The estimated rates of progression to AP/BC from yrs 1 through 7 are 1.5, 2.8, 1.6, 0.9, 0.5, 0, and 0.4%, respectively, with one pt progressing to AP/BC between yrs 6 and 7. Yearly event rates are 3.3%, 7.5%, 4.8%, 1.7%, 0.8%, 0.3% and 2% (5 events occurred in the 7th yr: 3 unconfirmed loss of MCyR, 2 deaths). Of the 456 pts who achieved CCyR, 79 (17%) subsequently lost CCyR; 25 remained on IM (19 pts regained CCyR, of whom 6 responded to an increase in IM dose; 6 pts remained in MCyR without dose escalation). A total of 15 pts (3%) who achieved CCyR on IM progressed to AP/BC during study treatment, typically during the 1st year after achievement of CCyR; 3 CCyR pts progressed to AP/BC after the 2nd year. A total of 332 (60%) pts remain on IM on protocol at the 7-yr data cut-off. Reasons for discontinuation or crossover include: 5% adverse events/safety, 15% lack of efficacy/progression, 3% bone marrow transplant, 2% death, and 15% other (protocol violation, withdrawal of consent or lack of renewal of consent, lost to follow-up, administrative) reasons. Between yrs 6 and 7, 17 pts (3%) discontinued IM for the following reasons: adverse events (n=3), death (n=2; 1 CML-related), unsatisfactory therapeutic effect (n=7; 1 progression to AP/BC, 4 unconfirmed loss of MCyR, 2 unconfirmed loss of CCyR), protocol violation (n=1), and withdrawal of consent (n=4). Molecular response (MR) assessment was required per the IRIS protocol only in pts who had achieved CCyR. However, MR was measured routinely in 98 pts treated in Australia/New Zealand and Germany (sub-study) at baseline and every 3 mo through 72 mo, and other sites contributed assessments if available. Of the total IRIS IM cohort, 476 pts had at least one PCR measurement. MMR was defined as a ratio of BCR-ABL/control transcripts of ≤ 0.1% according to the International Scale.

Table 1: MR over time: BCR-ABL/control gene transcript levels (as % of available samples)

	All available samples					Sub-study samples	
Time-points (mo)	n	>10%	> 1.0 - ≤10%	> 0.1 - ≤ 1.0%	≤ 0.1% (MMR)	n	≤ 0.1% (MMR)
3	174	25%	39%	24%	13%	87	8%
6	258	15%	17%	35%	33%	86	28%
12	305	9%	12%	30%	50%	81	47%
18	253	6%	10%	19%	65%	70	63%
48	238	6%	9%	10%	75%	66	82%
60	273	3%	4%	8%	85%	71	90%
72	210	2%	3%	9%	86%	57	88%

The MMR rates at 12 and 48 mo for all available samples are consistent with the reported rates of 53% and 80%, respectively, noted in a subset of pts with CCyR (Druker et al, NEJM, 2006) and similar to the unselected sub-study data. Additionally, MMR responses at 12 mo are similar to the recently reported TOPS trial (Cortes et al, EHA 2008). Between yr 6 and 7, serious adverse events suspected to be related to IM were reported in 9 pts, resulting in treatment discontinuation in 3 pts. No new safety issues were identified. Conclusions: Responses with IM therapy remain durable with estimated 7 yr rates of FFP to AP/BC 93%, EFS 81%, and OS 86%. Only 1 patient progressed between yrs 6 and 7. The safety profile is unchanged and confirms a favorable risk-benefit ratio in CML-CP. Long-term follow-up of pts who continue to respond to IM demonstrate an MMR rate of 85-90% at 5-6 years. These results demonstrate increasing suppression of CML over time in patients who continue to receive imatinib.

2.7. **[187] Is It Possible to Stop Imatinib in Patients with Chronic Myeloid Leukemia? An Update from a French Pilot Study and First Results from the Multicentre « Stop Imatinib » (STIM) Study.** Mahon *et al*. Imatinib (IM) has greatly improved survival rates in chronic myeloid leukemia (CML). However, all patients (pts) must continue treatment for an unknown period of time. A pilot study of the first

pts who discontinued IM therapy was previously reported (Rousselot et al. Blood 2007;109:58–60). The new, multicentre « Stop Imatinib » (STIM) study was started in July 2007. The aim of this study is to evaluate in a larger cohort the persistence of complete molecular remission (CMR) after stopping IM, and to determine the factors that could influence the persistence of CMR. The criteria for inclusion were IM treatment for at least 3 years and sustained CMR. Sustained CMR was defined as BCR-ABL/ABL levels below a detection threshold corresponding to a 5-log reduction (undetectable signal using RQ-PCR) for at least 2 years. Molecular relapse, defined as RQ-PCR positivity, was taken into account if confirmed in two successive assessments. In cases of molecular relapse, pts were re-treated with IM at 400 mg dailyIn the pilot study, 7 out of 15 pts relapsed within 6 months, but CMR was re-attained in all cases after IM was re-started. The other 8 pts (4 male, 4 female) are still in CMR, with a median follow up of 37 months (range 26–49 months) after IM discontinuation. All pts were pre-treated with interferon-alpha (IFN) and most responded to IFN before IM treatment. The STIM study included 50 pts from 18 centres (20 male, 30 female), with a median age of 62 years (range 32–81 years). Of these, 25 pts had received no pre-treatment with IFN. By July 2008, 34 pts had a follow up ≥ 6 months. Eighteen pts relapsed within the first 6 months: 3 pts in month 2 (M2), 8 pts in M3, 4 pts in M4, and 3 pts in M5. One patient relapsed after more than 6 months (M8). Among the 19 pts who relapsed, 11 were not IFN pre-treated and 8 were IFN pre-treated (relapse rate 44% vs 32%). Ten IFN pre-treated pts with follow up ≥ 6 months have not relapsed (M12 in 2 pts, M10 in 5 pts, M8 in 1 pt, M7 in 2 pts), and 5 pts with follow up ≥ 6 months who were not IFN pre-treated have not relapsed (M12 in 1 pt, M10 in 1 pt), M8 in 1 pt, M6 in 2 pts). These studies confirm that CMR can be sustained after discontinuation of IM, particularly in pts pre-treated with IFN with a long follow-up (pilot study). Among pts in the STIM study who were not pre-treated with IFN, more than half have not relapsed, and 20% have reached a follow-up ≥ 6 months and not relapsed. Updated data will be presented but we conclude that it is possible to stop treatment in pts with sustained CMR, even in those treated with IM as a single agent.

NOTES ON PRESENTATION

In an initial pilot study one patient has gone for 4 years with no evidence of recurrent disease. 15 initially. CMR for more than 2 years. 8 still in CMR. 30 to 49 months follow up mean 39.

STIM study. CMR for at least 2 years. 69 patients in 22 centres. 60 patients wih a follow up of more than 1 month. 31 previously treated with IFN, 29 de novo patients. Median follow up in only 5 months. 49 patients had more than 6 months 27 patients relapsed in first 6 months. 46% chance of being in CMR at 6 months. All patients are sensitive after imatinib rechallenge. IFN may not make the difference…

2.8. **[188] Epidemiological Study on Survival of Chronic Myeloid Leukemia (CML) and Ph+ Acute Lymphoblastic Leukemia (ALL) Patients with T315I Mutation. Final Analysis.** Nicolini *et al.* The BCR-ABL T315I mutation is one of the major mechanisms of resistance to tyrosine kinase inhibitors (TKIs). Limited data have suggested that patients harboring a T315I mutation have poor outcomes. The objectives of this study were to estimate overall (OS) and progression-free survival (PFS) for CML in chronic (CP), accelerated (AP), or blastic (BP) phase, and Ph+ ALL patients who developed a T315I mutation; and describe the treatment pattern after T315I detection. Methods: This was a retrospective, multi-center observational study. Eligible patients included CML and Ph+ ALL patients who developed T315I mutation between 1999 and 2008. The medical records of 222 patients from 9 countries (France, Italy, Korea, USA, Germany, Singapore, Denmark, UK and Japan) were abstracted, and Kaplan-Meier plots and Cox proportional hazard models were used for survival analysis. Results: Median age at T315I detection was 54 (range, 18-84) years; 57% were male; 75% were Caucasian and 22% were Asian. Before T315I detection, 97% patients received imatinib (25% as a 1st line) and 50% received second generation TKIs. 16% of patients had other mutations detected before T315I detection. The median time between TKI treatment start and T315I detection was 29 months for CP, 15 for AP, 6 for BP, and 9 for Ph+ ALL. After T315I detection, 56% patients received second generation TKIs (30% started after T315I detection), 39% received hydroxyurea (33% started after T315I detection), 35% received imatinib (13% started after T315I detection), 26% received cytarabine, 21% received investigational drugs including 11% MK-0457, 17% underwent stem cell transplantation, and 6% received interferon alpha (5% started after T315I detection). At the time of T315I detection, T315I formed the predominant clone in 87% of patients; 23% had additional mutations detected (11% of these P-loop mutations). OS and PFS from T315I mutation detection are summarized in Table 1. In a preliminary analysis, the following covariates were associated with worse OS in Cox proportional hazard model (adjusted hazard ratio, 95% confidence interval): older age (by median, 2.30, 1.04-5.09) in Ph+ ALL patients, female gender in BP (1.73, 0.96-3.10); worse performance status in Ph+ ALL (1+ vs. 0; 2.18, 1.02-4.68); and detection of T315I by direct

sequencing (vs. other methods) in AP (3.03, 0.89-10.29) and Ph+ ALL (2.33, 1.06-5.12). The effect of different treatments on OS will be available at the time of presentation. Conclusion: These results confirm that survival of patients harboring a T315I mutation is dependent on the disease phase at T315I detection. No clear treatment pattern after T315I detection was observed. Age, gender, performance status, and techniques used for T315I detection might be important prognosis factors affecting OS across different phases of CML and Ph+ ALL.

3 Chronic myeloid leukemia - stem cell biology and eradication [abstracts 189 - 196]

3.1. **[189] Activation of PP2A by FTY720 Inhibits Survival and Self-Renewal of the Ph(+) Chronic Myelogenous Leukemia (CML) CD34+/CD38- Stem Cell through the Simultaneous Suppression of BCR/ABL and BCR/ABL–independent Signals.** Neviani *et al.* CML is a clonal disorder of the pluripotent hematopoietic stem cell characterized by the sustained kinase activity of the BCR/ABL oncoprotein. We reported that the BCR/ABL-dependent and SET-mediated inhibition of protein phosphatase PP2A tumor suppressor activity is essential for the leukemogenic potential of CD34+ CML bone marrow progenitors, as molecular and pharmacologic restoration of PP2A inhibits the activity of BCR/ABL and that of several important regulators of cell survival/proliferation, thus resulting in marked apoptosis, impaired clonogenic potential and in vivo leukemogenesis of imatinib/dasatinib-sensitive and -resistant Ph(+), but not normal, CD34+ blasts and/or BCR/ABL+ mouse marrow progenitors. Here we show that SET-dependent suppression of PP2A activity is a common feature of Ph(+) progenitors (CMP and GMP) and imatinib/dasatinib-insensitive CD34+/CD38- BCR/ABL+ (n=3) stem cells but not of the equivalent cell fractions from healthy individuals (n=3). To determine the biological importance and therapeutic implications of impaired PP2A activity in Ph(+) stem cells, we evaluated by clonogenic, CFC/replating, LTC-IC and CFSE-mediated cell division-tracking assays, the effects of FTY720 (2.5 mM), a PP2A activator currently in phase III trials for MS patients, and lentiviral-mediated ectopic PP2Ac expression on survival and self-renewal of BCR/ABL+ stem/progenitor cells isolated from bone marrow of CML blast crisis patients (ntot=8; Ph1≥90%) and/or SCL-tTA-BCR/ABL transgenic animals (ntot=10). FTY720 treatment (2.5-5mM) severely suppressed the clonogenic potential of CD34+/CD38- and CD34+/CD38+/CD45RA-/+ CML stem/progenitor cells. Accordingly, self-renewal and long-term repopulating potential of CML leukemic stem cells was markedly impaired by pharmacologic PP2A reactivation. In fact, the CFC output of LTC-IC cultures (6 weeks) deriving from FTY720-treated (2.5 mM; 72h) Ph(+) CD34+ cells was more than 95% inhibited if compared to that of LTC-IC cultures from untreated CML cells. By contrast, imatinib (5 mM) and dasatinib (200 nM) treatment led to a 3.5 and 5-fold increase in CFC output, respectively. Consistent with the ability of FTY720 to impair self-renewal of CML stem cells, a 50-90% reduction of the CFSEMAX/quiescent cell population was observed in CFSE-stained CD34+ CML cells treated for 6-9 days with FTY720. Notably, FTY720 did not exert any significant effect on CFSE-stained CD34+ cells from healthy individuals whereas, as expected, imatinib (5 mM) and dasatinib (200 nM) treatment led to a 22% and 27% increase in CFSEMAX CML cells, respectively. Interestingly, only FTY720 triggered apoptosis of CFSEMAX CML cells (41% Annexin V+ cells) although BCR/ABL activity (phospho-ABL intracellular flow-cytometry staining) in CFSEMAX cells was efficiently inhibited by FTY720, Imatinib and dasatinib, suggesting that BCR/ABL-independent PP2A-regulated signals control the survival and self-renewal of CML stem cells. Indeed, lentiviral-driven PP2Ac-overexpression as well as treatment with FTY720, but not imatinib, significantly decreased (40-90% reduction) CFC/serial replating efficiency, colony size and percentage of CFSEMAX fraction (66-96% reduction) of Lin-/Sca+/Kit+ (LSK) cells isolated from bone marrow and spleen of leukemic SCL-tTA-BCR/ABL mice. Mechanistically, the detrimental effect of PP2A activation on survival and self-renewal of CML stem cells might depend on the ability of PP2A to inactivate b-catenin that, reportedly, is a PP2A target essential for the self-renewal of the CML blast crisis GMP progenitors. In fact, immunoblotting, direct immunofluorescence and LET/TCF luciferase assays showed that ectopic PP2Ac expression and/or FTY720, but not imatinib, treatment leads to inactivation/degradation of nuclear b-catenin in BCR/ABL+ primary mouse LSK and/or 32D-BCR/ABL cells. Altogether our data not only highlight the importance of PP2A inactivation for survival and self-renewal of CML stem cells but also suggest the existence of BCR/ABL-independent, PP2A-sensitive and b-catenin-mediated signals that may account for resistance of CML quiescent stem cells to tyrosine kinase inhibitor monotherapy. Thus, FTY720 treatment has the potential to eradicate CML by efficiently targeting both stem and progenitor Ph(+) cells regardless of their degree of sensitivity to imatinib and dasatinib.

3.2. **[190] Suppression of CML Progenitor but Not CML Stem Cell Growth Requires Dual Inhibition of BCR-ABL and KIT.** Corbin *et al*. Chronic myeloid leukemia (CML) is caused by the tyrosine kinase BCR-ABL and inhibition of BCR-ABL by imatinib correlates with clinical response. In addition to BCR-ABL, imatinib inhibits c-ABL, ARG, PDGFR, c-FMS, LYN, KIT and possibly other kinases. It is presently unclear whether there are therapeutic benefits derived from simultaneous inhibition of BCR-ABL and non-BCR-ABL targets within CML cells. KIT is a receptor tyrosine kinase expressed by both normal and leukemic hematopoietic progenitors. Its ligand, stem cell factor (SCF), supports proliferation of CML progenitors in the absence of other cytokines and thus KIT signaling in conjunction with BCR-ABL signaling may be central to leukemogenesis. We hypothesized that the capacity of imatinib to inhibit KIT may contribute to its efficacy in CML. In this study, we use a panel of sole BCR-ABL, sole KIT and dual BCR-ABL/KIT inhibitors to explore: 1) the requirement for BCR-ABL and KIT signaling in the survival and growth of primary CML stem and progenitor cells and 2) the degree to which dual BCR-ABL/KIT inhibition by imatinib contributes to its efficacy in CML. We evaluated CFU-GM colony formation from newly diagnosed CML cells cultured in SCF, IL-3, and GM-CSF in the presence of the following BCR-ABL and KIT inhibitors: imatinib (dual BCR-ABL/KIT), PPY-A (sole BCR-ABL), SCF-blocking antibody K44.2 (SCF-block, sole KIT) and PPY-A+SCF-block (BCR-ABL+KIT). Enumeration of CFU-GM revealed minimal suppression by PPY-A (30%) and somewhat more significant suppression by SCF-block (50%). Maximal colony suppression achieved during treatment with imatinib (80%) could only be reproduced under conditions of simultaneous BCR-ABL and KIT inhibition using PPY-A+SCF-block ($p=0.4$ relative to imatinib) or PPY-A upon removal of SCF ($p=0.5$ relative to imatinib). Removal of IL-3 or GM-CSF did not significantly impact the growth of CFU-GM under conditions of sole BCR-ABL inhibition. We additionally cultured CML progenitors on human or murine stroma for 1-3 weeks under conditions of BCR-ABL and/or KIT inhibition and evaluated colony forming cell (CFC) growth. Neither sole BCR-ABL nor sole KIT inhibition suppressed growth of week 1 or week 3 CFC to the degree of dual inhibition by either imatinib or PPY-A+SCF-block. Our findings indicate that targeted therapy aimed at BCR-ABL without simultaneously targeting KIT may not be as effective at eliminating proliferative progenitors that are largely responsible for the expansion of leukemic cells. To evaluate whether KIT supports CML stem cell survival in a similar manner, we performed 6-week LTC-IC on murine stroma with BCR-ABL and KIT inhibitors. Sole BCR-ABL inhibition was more effective at blocking CML stem cell growth than progenitor cell growth (98% versus 71% suppression respectively) and KIT inhibition did not affect this population. The requirement of dual BCR-ABL and KIT inhibition to suppress primary CML cell growth was therefore cell context dependent and restricted to multipotent proliferative progenitors and CFU-GM. We conclude that 1) despite the presence of BCR-ABL in all cell types associated with the malignant clone, survival depends on both oncogenic and physiological signaling pathways. 2) Survival of CML stem cells, as seen in patients harboring residual disease, is independent of KIT. This may offer a partial explanation of why imatinib is less effective against this population than KIT-dependent progenitors. 3) The degree to which specific oncogene-independent pathways contribute to survival impacts the efficacy of targeted therapy.

3.3. **[191] Leukemic Stem Cells and Progenitors Demonstrate Impaired Interaction with the Hematopoietic Microenvironment in Vivo in An Inducible Murine Model of Chronic Myelogenous Leukemia.** Sengupta *et al*. Chronic myelogenous leukemia (CML) is a stem cell malignancy induced by p210 BCR-ABL and characterized by myeloproliferation in BM and egression of leukemic stem cells and progenitors (LSC/P) to extramedullary sites. Persistence of BCR-ABL+ HSC in patients under Imatinib suggests inhibition of ABL-kinase alone is not sufficient to eliminate the LSC/P. One of the major hallmarks of CML induced by signaling downstream BCR-ABL is the loss of control of the hematopoietic microenvironment on LSC/P. Expression of p210 BCR-ABL has been associated with loss of adhesion to the bone marrow, impaired migration in response to CXCL12 and decreased retention in the BM. In order to study the putative LSC/P niches in steady-state chronic-phase leukemia, we have analyzed the ability of LSC/P to proliferate and get retained in the bone marrow (BM) in an inducible model of CML. Binary transgenic SCL-tTA/TRE-BCR-ABL mice (Koschmieder S et al., Blood 2005) express p210 BCR-ABL in LSC/P upon doxycycline withdrawal (CML mice). Induced myeloproliferation was associated with activation of the downstream signaling effectors CrkL and p38-MAPK and expansion of circulating (Table 1) and splenic LSC/P but not in BM, suggesting massive LSC/P egression from the marrow (Table 2). Proliferation analysis showed that myeloid expansion in the spleen was secondary to increased cycling of Lin-Sca1+c-Kit+ (LSK) cells (3.1-fold increase in S-phase cells, $P<0.05$), but not in Lin-/c-Kit+ (LK) population, compared with the control spleens. In agreement with the LSC/P BM content data, the frequency of BM-derived LSK and LK cells incorporating BrdU in CML and in control mice remained similar, suggesting a specific egression of LSC/P from the BM to extramedullary sites. To test whether this model truly represented a model of BM LSC/P egression, we compared the splenic and BM LSC/P compared with their controls regarding their adhesion molecule expression, interaction with the

hematopoietic microenvironment (HM) and homing to the overall marrow cavity and endosteal space. Splenic, but not BM-derived, LSK and LSK CD34+ ST-HSCs had increased cell surface expression of CD44 compared to controls (1.35 fold, P=0.006 and 1.23 fold, P<0.05 respectively) and decreased expression of L-selectin (8.7 fold, P<0.05) while expression of CXCR4, α4β1 and α5β1 integrins remain similar in bone marrow and splenocytes from CML and control mice. CML BM progenitors also showed 18-fold reduced adhesion to fibronectin and 1.4-fold increased migration towards CXCL12 compared to control BM progenitors. Myeloproliferative disease was transplantable into non-transgenic littermates and homing of CML BM progenitors was increased (4.3 fold, P<0.005) in myeloablated littermate recipient BM. However, lineage-negative leukemic BM-derived cells which had increased homing in BM of recipient mice had an impaired ability to migrate to the BM endosteal space compared with their littermate controls (control: 31 ± 18% vs CML mice: 17.6 ± 17%), suggesting an specific impairment to lodge in specialized anatomically-defined hematopoietic "niches". Altogether, this murine model may represent an adequate in vivo system to analyze the ability of p210 BCR-ABL-expressing LSC/P to interact with BM niches and study the control of the hematopoietic microenvironment on LSC/P survival, proliferation and retention.

3.4. **[192] Mechanisms Generating free Radicals in CML Stem/Progenitor Cell Populations Causing DNA Damage and Genomic Instability.** Nieborowska-Skorska *at al.* BCR/ABL kinase is the founding member of a family of oncogenic tyrosine kinases (OTKs) also including TEL/JAK2, TEL/PDGFR, TEL/ABL, and JAK2V617F, which induce myeloproliferative disorders (MPDs). BCR/ABL transforms hematopoietic stem cells (HSCs) to induce chronic myelogenous leukemia in chronic phase (CML-CP), which eventually evolves into fatal blast crisis (CML-BC). CML is a stem cell-derived but progenitor-driven disease. In CML-CP, leukemia stem cells (LSCs) and leukemia progenitor cells (LPCs) reside in the CD34+CD38- and CD34+CD38+ populations, respectively, whereas in CML-BC, LSCs are also found in the CD34+CD38+ population. In addition, CD34+ CML cells belong to either proliferative or quiescent populations; the latter of which responds poorly to the ABL kinase inhibitors. BCR/ABL kinase stimulates genomic instability causing imatinib-resistant point mutations in the kinase domain and additional chromosomal aberrations associated with progression to CML-BC (Oncogene, 2007). Since genomic instability usually results from enhanced DNA damage, we investigated the mechanisms responsible for "spontaneous" DNA damage in cells transformed by BCR/ABL and other OTKs. Much endogenous DNA damage arises from free radicals such as reactive oxygen species (ROS) and/or reactive nitrogen species (RNS). We showed that CD34+ stem/progenitor CML cells contain higher levels of ROS (superoxide anion = •O2-, hydrogen peroxide = H2O2 and hydroxyl radical = •OH) and RNS (nitric oxide = NO•) than CD34+ cells from normal donors (CML-BC>CML-CP>Normal). Moreover, ROS levels were elevated in CD34+CD38- and CD34+CD38+ sub-populations isolated from CML-BC and CML-CP patients in comparison to the corresponding cells from healthy donor. In addition, both proliferative and quiescent CD34+ CML cell sub-populations contained more ROS than their normal counterparts. Interaction with the stromal cells further elevated ROS levels in BCR/ABL-positive cells. Higher ROS/RNS levels induced more oxidative/nitrative DNA lesions, such as 8-oxoG and DNA double-strand breaks (DSBs), in CML-CP cells resulting in induction of point mutations in BCR/ABL kinase causing imatinib resistance and accumulation of chromosomal aberrations characteristic of CML-BC. In addition, cells transformed by other OTKs also displayed elevated ROS/RNS and oxidative/nitrative DNA damage, implicating their role in malignant progression of MPDs. Our previous studies showed that elevated levels of oxidative DNA damage in OTK-transformed cells could be diminished by scavenging of ROS with N-acetyl-cysteine and vitamin E, which reduced the frequency of imatinib-resistant BCR/ABL point mutants and chromosomal aberrations in leukemia cells cultured in vitro and growing in SCID mice (Blood, 2006; Leukemia, 2008). These studies highlighted the importance of identification of the sources of free radicals in CML and other MPDs. We found that elevated levels of ROS in BCR/ABL-transformed cell lines and CD34+ CML cells were generated by three major mechanisms: NADPH oxidase (NOX) complexes containing NOX1 and/or NOX2, complex III of the mitochondrial respiratory chain (MRC), and 5-lipoxygenase (LOX). In addition, inducible nitric oxygen synthase (iNOS) produced RNS in leukemia cells. Using selective inhibitors of NOX, MRC, LOX and iNOS we estimated the contribution of these pathways to accumulation of free radicals causing oxidative/nitrative DNA damage in CML cells. In summary, BCR/ABL kinase-dependent elevation of ROS/RNS depends on several mechanisms, which are now targeted to determine their actual role in genomic instability in CML.

3.5. **[193] Differentially Expressed and Novel Transcripts in Highly Purified Chronic Phase CML Stem Cells.** Zhao *at al.* The chronic phase of CML is sustained by rare BCR-ABL+ stem cells. These cells share many properties with normal pluripotent hematopoietic stem cells, but also differ in critical ways that alter their growth, drug responsiveness and genome stability. Understanding the molecular mechanisms underlying the biological differences between normal and CML stem cells is key to the

development of more effective CML therapies. To obtain new insights into these mechanisms, we generated Long Serial Analysis of Gene Expression (SAGE) libraries from paired isolates of highly purified lin-CD34+CD45RA-CD36-CD71-CD7-CD38+ and lin-CD34+CD45RA-CD36-CD71-CD7-CD38- cells from 3 chronic phase CML patients (all with predominantly Ph+/BCR-ABL+ cells in both subsets) and from 3 control samples: a pool of 10 normal bone marrows (BMs), a single normal BM and a pool of G-CSF-mobilized blood cells from 9 donors. In vitro bioassays showed the CD34+CD38+ cells were enriched in CFCs (CML: 3-20% pure; normal: 4-19% pure) and the CD34+CD38- cells were enriched in LTC-ICs (CML: 0.2-26% pure; normal: 12-52% pure). Each of the 12 libraries was then sequenced to a depth of ~200,000 tags and tags from libraries prepared from like phenotypes were compared between genotypes using DiscoverySpace software and hierarchical clustering. 1687 (355 with clustering) and 1258 (316 with clustering) transcripts were thus identified as differentially expressed in the CML vs control CD34+CD38- and CD34+CD38+ subsets, respectively. 266 of these transcripts (11 with clustering) were differentially expressed in both subsets. The differential expression of 5 genes (GAS2, IGF2BP2, IL1R1, DUSP1 & SELL) was confirmed by real-time PCR analysis of lin-CD34+ cells isolated from an additional 5 normal BMs and 11 CMLs, and lin-CD34+CD38- cells from an additional 2 normal BMs and 2 CMLs (with dominant Ph+ cells). GAS2 and IL1R1 transcript levels were correlated with BCR-ABL transcript levels in both primitive subsets, and predicted differences in expression of IL1R1 and SELL were apparent within 3 days in CD34+ cord blood cells transduced with a lenti-BCR-ABL-IRES-GFP vs a control lenti-GFP vector (n=3). These findings support a direct role of BCR-ABL in perturbing the expression of these 3 genes. Further comparison of the meta CD34+CD38- and CD34+CD38+ CML cell libraries with most publicly accessible SAGE data revealed 69 novel tags in the CD34+ CML cells that correspond to unique but conserved genomic sequences. Nine of these were recovered by 5'- and 3'- RACE applied to cDNAs pooled from several human leukemic cell lines. These results illustrate the power of SAGE to reveal key components of the transcriptomes of rare human CML stem cell populations including transcripts of genes not previously known to exist. Continuing investigation of their biological roles in primary CML cells and primitive BCR-ABL-transduced human cells offer important strategies for delineating their potential as therapeutic targets.

3.6. **[194] Persistence of Leukemia Stem Cells in Chronic Myelogenous Leukemia Patients in Complete Cytogenetic Remission on Imatinib Treatment for 5 Years.** Chu *et al.* Imatinib mesylate (IM) treatment results in marked reduction in burden of leukemia cells in chronic myelogenous leukemia (CML) patients, as indicated by achievement of complete cytogenetic remission and major reduction in Bcr-Abl transcript levels on Q-PCR analysis. However patients treated with IM alone without prior interferon treatment appear to invariably relapse on discontinuation of IM treatment. In addition we and others have shown that residual Bcr-Abl+ progenitors persist in IM-treated CML patients following achievement of CCR. These observations suggest that despite its remarkable activity in CML, IM fails to eliminate all malignant stem and progenitor cells in CML patients. However our previous studies were conducted on patients within the first year or two of IM treatment, whereas recent studies have indicated that Bcr-Abl levels continue to decline on Q-PCR analysis with continued IM treatment. This together with the decreasing rate of disease relapse observed after 3 years of IM treatment raises the possibility that prolonged IM treatment may cause depletion of residual CML stem cells. In this study we investigated whether prolonged IM treatment was associated with a reduction in Bcr-Abl+ stem and progenitor cells. We evaluated 14 CML patients followed at our center who were in CCR, had been treated with IM for at least 4 years, and from whom multiple cryopreserved bone marrow samples were available for study. Bone marrow mononuclear cells (MNC) were thawed, CD34+ cells were selected by immunomagnetic columns, and CD34+CD38+ (38+) committed progenitors and CD34+CD38- (38-) stem/primitive progenitor cells were isolated by flow cytometry sorting. Q-PCR analysis of Bcr-Abl and Bcr transcript levels was performed on RNA isolated from MNC, 38+ and 38- cells and Bcr-Abl levels were reported as the ratio of Bcr-Abl to Bcr. Bcr-Abl levels in MNC were 0.010±0.005, 0.011± 0.005and 0.013±0.005 at 3, 4 and 5 years. We observed that Bcr-Abl levels were higher in both 38+ and 38- cells in comparison with levels in MNC. A gradual decline in Bcr-Abl levels in 38+ cells was seen (0.285±0.185 at 3 years, 0.121±0.056 at 4 years, and 0.071±0.028 at 5 years). In contrast high Bcr-Abl levels were maintained in the 38- fraction despite continued IM treatment (0.162±0.086 at 3 years, 0.116±0.041 at 4 years, and 0.361±0.107 at 5 years). In contrast to IM-treated patients, Bcr-Abl transcripts were not detected in MNC and CD34+ cells from BM of CML patients who had received allogeneic hematopoietic cell transplants (n=5). To further investigate whether malignant stem cells persisted after prolonged IM treatment, MNC from 5 of the patients described above were transplanted by tail vein injection into sublethally irradiated NOD/SCID-IL2Rγ-chain knockout (NSG) mice. High levels of human cell engraftment were observed 4-5 weeks after injection, and Q-PCR analysis revealed high levels of Bcr-Abl expression in engrafted cells from 4 of 5 patients, confirming the presence of Bcr-Abl+ cells with NOD/SCID mouse repopulating

capacity. In conclusion, our results clearly demonstrate the persistence of Bcr-Abl+ stem cells in the BM of CML patients in prolonged remission after 5 years of IM treatment. The observed persistence of leukemia stem cells raises the concern that patients remain at risk for relapse on drug discontinuation or through acquisition of IM resistance. The assays described here may have considerable utility for evaluating and monitoring the effects of experimental treatment strategies directed against residual CML stem cells.

3.7. **[195] Selective Anti-Leukemia Targeting of the Interaction Between BCR/ABL and Mammalian RecA Homologs.** Slupianek *et al.* We showed before that cells transformed by BCR/ABL and other fusion tyrosine kinases (FTKs) such as TEL/ABL, TEL/JAK2 and TEL/PDGFR, inducing chronic myeloproliferative disorders (MPDs), and CD34+ chronic myeloid leukemia (CML) stem/progenitor cells from chronic phase (CML-CP) and blast crisis (CML-BC) contain an excess of DNA double-strand breaks (DSBs) induced by reactive oxygen species (ROS) and genotoxic stress [Blood, 2005; Cell Cycle, 2006; DNA Repair, 2006; Cancer Res., 2008]. Recent studies also revealed that CD34+CD38- CML-CP and CML-BC stem cell-enriched populations seem to display more DSBs than normal counterparts as measured by gamma-H2AX foci formation on DNA. Elevated levels of DSBs were also observed in leukemia cells expressing imatinib-resistant BCR/ABL kinase mutants. DSBs may cause apoptosis if not repaired or chromosomal aberrations if repaired unfaithfully. Numerous ROS- and radiation- induced DSBs are not lethal for BCR/ABL-positive leukemia cells; instead, they induce chromosomal instability implicating enhanced, but unfaithful repair [Leukemia, 2008]. The previous report [Mol.Cell, 2001] and ongoing studies demonstrated that BCR/ABL kinase (non-mutated and imatinib-resistant mutants) modulates expression of the mammalian RecA homologs RAD51, RAD51B, RAD51C, RAD51D, XRCC2 and XRCC3, which are responsible for homologous recombination repair (HRR) of DSBs. RAD51 plays a key role in HRR in cells transformed by BCR/ABL and other FTKs [Mol.Cell, 2001; Mol.Cell.Biol., 2002]. BCR/ABL stimulates the expression of, interacts with and phosphorylates RAD51 on Y315, which is located in a critical fragment of RAD51 essential for its filament formation on DNA. Accordingly, our recent results indicated that BCR/ABL-mediated RAD51[Y315] phosphorylation appears to be important for nuclear RAD51 foci formation in response to DNA damage. In addition to RAD51, BCR/ABL interacts directly with and phosphorylates RAD51B and XRCC2, but not other RecA homologs. Altogether, it appears that BCR/ABL can deregulate the expression and phosphorylation of some RecA homologs, which may have a significant impact on the efficiency and fidelity of DSB repair resulting in protection from apoptosis and chromosomal instability. Therefore, disassembly of BCR/ABL from RecA homologs should reduce the capability of CML cells to repair numerous ROS-induced DSBs and eventually trigger apoptosis. Based on this hypothesis we investigated the mechanisms of association between BCR/ABL and RecA homologs. Interactions between BCR/ABL and RAD51 or RAD51B depend on the proline- rich (PP) regions of RAD51 and RAD51B, and the SH3 domain and SH2-catalytic domain (SH2-CD) linker of BCR/ABL, which form a pocket binding the PP regions. Disruption of the PP regions of RAD51 by P-L amino acid substitutions (PP-LL mutants) abrogated direct interaction with the BCR/ABL SH3-SH2-CD pocket. On the other hand, single amino acid substitutions in the BCR/ABL SH3-SH2-CD pocket, which eliminated its capability of binding the PP regions, prevented complex formation with RAD51 and RAD51B. In addition, RAD51 and RAD51B may interact with members of the BCR/ABL proteome such as Grb2 and Shc (RAD51 and RAD51B), and c-CrkL (only RAD51B), but not Gab2 and c-Cbl. 32Dcl3 murine hematopoietic cells expressing BCR/ABL SH3-SH2-CD pocket mutant, where single amino acid substitutions disrupted its direct interaction with RAD51, displayed a slower proliferation rate and responded poorly to genotoxic stress despite intact kinase activity in comparison to cells transformed with non-mutated BCR/ABL. Interestingly, expression of RAD51 PP-LL mutant eliminated BCR/ABL-transformed leukemia cells, without any toxic effect on normal counterparts. These results suggest that the interaction between BCR/ABL and RAD51 may be targeted for selective elimination of leukemia cells and/or suppression of genomic instability. To test this hypothesis we are employing the peptide aptamer strategy targeting RAD51 PP regions in CD34+ cells obtained from imatinib-sensitive and imatinib-resistant CML patients and healthy volunteers in vivo and in vitro. In summary, we hypothesize that mechanisms regulating the association of BCR/ABL with RAD51 and other mammalian RecA homologs may be explored for the planning of more effective anti-tumor modalities.

3.8. **[196] Reduced Activity of the OCT-1 Protein in Primitive CML Cells: A Likely Determinant of Stem Cell Resistance in Imatinib Treated CML Patients.** Engler *et al*. Despite cytogenetic and molecular remissions, residual chronic myeloid leukemia (CML) cells persist in the primitive CD34+ compartment in the majority of imatinib treated patients. It has been demonstrated that CD34+ CML cells have a reduced sensitivity to imatinib induced apoptosis. Factors which may contribute to this reduced sensitivity are reduced dependence on BCR-ABL, an increase in BCR-ABL transcripts, increased

expression of efflux proteins or decreased expression of drug influx proteins. Our previous studies show that a patient's intrinsic sensitivity to imatinib-induced kinase inhibition (IC50) is related to the intracellular uptake and retention (IUR) of imatinib in peripheral blood mononuclear cells. The organic cation transporter 1 (OCT-1) is the major active influx transporter for imatinib in these cells, and the functional activity of this protein (determined using functional inhibition of OCT-1, in the IUR assay) directly correlates with molecular response to imatinib. In the present study we investigated the role that OCT-1 plays in stem cell resistance to imatinib. Primitive CD34+ and mature CD34- cells were isolated from CML patients and normal individuals using magnetic cell sorting. CML CD34+ cells had a significantly lower IURimatinib than that of CML CD34- cells (Table 1). The addition of the OCT-1 inhibitor, prazosin (100μM), eliminated this difference in IUR (Table 1), indicating that variation in IURimatinib is due to variation in the functional activity of the OCT-1 protein. In addition, the OCT-1 Activity (Table 1) and OCT-1 mRNA expression (expressed as % of BCR: mean CD34+=0.25; CD34-=4.9, p=0.040, n=10) was significantly lower in CML CD34+ cells compared with CML CD34- cells. These differences in IURimatinib and OCT-1 activity between CD34+ and CD34- cells were not observed in normal individuals (Table 1), suggesting this phenomenon is specific to leukemic cells. Furthermore, we isolated the more primitive compartment, CD34+38- cells and less primitive CD34+38+ cells in 4 CML patients. The CD34+38- cells demonstrated a 13% reduction in IURimatinib and a 41% reduction in OCT-1 activity compared with CD34+38+ cells. These data suggest a reduced IUR mediated by low OCT-1 function and/or expression may play a role in the resistance of CML stem cells to imatinib.

Increased expression of efflux transporters of imatinib (i.e. ABCB1 and ABCG2) has been suggested as an important mechanism for drug resistance. The effect of an ABCB1 inhibitor (PSC833) and ABCG2 inhibitor (Ko143) was assessed in CD34+ cells from 3 CML patients, using the IUR assay. Neither of these drugs had any effect on the IURimatinib in CML CD34+ cells. Additionally the mRNA expression of ABCB1 did not differ between CML CD34+ and CD34- cells (expressed as a % of BCR: mean CD34+=33.7; CD34-=33.77, p=0.064, n=10). These data suggest that alterations in imatinib influx (via OCT-1) are more critical for development of stem cell resistance rather than differences in efflux. We have previously demonstrated that, unlike imatinib, the OCT-1 protein is not involved in nilotinib transport, as the addition of OCT-1 inhibitors does not alter IURnilotinib in patients. Assessing the IUR of nilotinib in CD34+ and CD34- CML cells reveals no significant difference between the two populations (Table 2). Additionally, the IURnilotinib is significantly higher than IURimatinib in CML CD34+ cells (Table 2). In summary, the reduced OCT-1 mediated uptake of imatinib in more primitive, CD34+ CML cells may result in inadequate kinase inhibition and contribute to stem cell resistance in CML. Since nilotinib uptake into CML CD34+ cells is not impaired in the same manner as imatinib, more substantial depletion of the primitive CML cells may be achieved.

4 Chronic myeloid leukemia - response and prognosis [abstracts 331 - 336]

4.1. **[331] The Initial Molecular Response of Chronic Phase CML Patients Treated with Second Generation ABL Inhibitor Therapy after Imatinib Failure Can Predict Inadequate Response and Provide Indications for Rational Mutation Screening.** Branford *at al.* Molecular analysis is recommended for monitoring patients (pts) with CML. For imatinib treated pts in chronic phase (CP), molecular analysis provides important prognostic information. A major molecular response (MMR, BCR-ABL ≤0.1% IS (international scale)) is associated with favourable progression free survival and is a primary endpoint of clinical trials. The 3 month (m) BCR-ABL level is predictive of MMR and almost all de-novo pts with values ≤1.0% IS subsequently achieve MMR. The second generation tyrosine kinase inhibitors nilotinib and dasatinib (2TKI) have demonstrated efficacy for CP pts who fail imatinib therapy due to resistance or intolerance. However, treatment failure associated with the presence of a limited spectrum of resistant mutations is evident. Furthermore, it has recently been suggested that failure to achieve a major cytogenetic response (MCR) by 12m defines inadequate response and these pts should be considered for alternative therapies (Cortes et al, Blood,2008,112,516). Pts in minor cytogenetic response or complete hematologic response at 12m had a projected 1 year progression rate of 17% compared to 3% for those with MCR at 12m. The value of molecular monitoring in the setting of 2TKI has not been defined in terms of the early prediction of response or emergence of resistant mutations. We monitored BCR-ABL levels and mutation status in 155 CP pts treated with nilotinib (n=73; 400mg BD) or dasatinib (n=82; ≥100mg (76/82 70mg BD)) after imatinib failure for a median of 18m (range (r) 3-36). The BCR-ABL level at 3m of 2TKI was highly predictive of subsequent MMR, P<0.0001 (Figure A). Similarly, the 3m BCR-ABL level was highly predictive of MCR, P<0.0001 (Figure B). Among pts with BCR-ABL >10% IS, those who failed to achieve at least 50% at 3m had a significantly lower probability of MCR compared to those between 10-50%: 11% vs 56% by 24m, P=0.003. These analyses were also

performed for pts with mutations at baseline (72/155, 46%) and for those without baseline mutations (83/155, 54%). The MMR and MCR rates based on the 3m BCR-ABL were still highly significant irrespective of the baseline mutation status, P<0.0001. We investigated factors associated with emergence of new mutations that have demonstrated a degree of resistance to 2TKI (2TKI resistant): T315I/A, F317L/I/V, V299L for dasatinib and T315I, Y253H, E255K/V, F359V/C (IC50 >150nM) for nilotinib. All pts with new mutations during dasatinib therapy (19/82, 23%) had one of the dasatinib 2TKI resistant mutations: T315I 9, F317L/I 6, V299L 4 pts. Mutations emerged in 15/73 (21%) nilotinib treated pts and were nilotinib 2TKI resistant in 11 pts (15%): T315I 5, F359V 4, Y253H 4, E255V 1 (2 pts had multiple mutations). 2TKI resistant mutations were detected at a median of 6m (r 1-24) and were more frequent in pts who already had a mutation at baseline compared to those without: 24/72 (33%) vs 6/83 (7%), P<0.0001. At the time of last molecular analysis 18 of 30 pts with new 2TKI resistant mutations had progressed, 7 had not progressed and the outcome was unknown for 5. Among pts with baseline mutations, the 3m BCR-ABL did not predict the emergence of 2TKI resistant mutations by 24m. Conversely, for pts without a baseline mutation the 3m BCR-ABL was predictive of emergent 2TKI resistant mutations when pts were divided into 2 groups: 1/53 pts (2%) ≤10% IS vs 5/30 pts (17%) >10% IS, P=0.02. In 16/30 pts (53%) with emergent 2TKI resistant mutations, BCR-ABL never fell below 10% IS. The rise in BCR-ABL associated with emergent mutations was minimal in these pts: median 2.2-fold and 6 pts had no change in BCR-ABL from baseline. This is a reflection of minimal response to 2TKI and hence minimal BCR-ABL reduction in these pts. The outcome is known for 13 of the 16 pts and 11/13 progressed. Regular mutation screening would be warranted in all pts with BCR-ABL >10% IS rather than upon a significant rise. The rise associated with emergent mutations when BCR-ABL was ≤10% IS was significantly higher: median 7.3-fold, P<0.0001. This degree of rise should be readily detected by serial analysis and would trigger mutation screening. In conclusion, BCR-ABL measured at 3m of 2TKI could predict response and for pts without baseline mutations it could predict the emergence of new mutations. All pts with BCR-ABL >10% IS are at risk of acquiring 2TKI resistant mutations and would benefit from regular mutation screening until BCR-ABL falls below 10% IS. Thereafter, a significant rise of >5-fold in BCR-ABL should trigger mutation screening.

NOTES ON PRESENTATION

155 total. 73 nilot 82 dasat. Achievement of MMR at 24 months is predicted by response at 3 months. 2nd gen inhibitors lumped together. Mutations emerged in 35 of 155 patient during therapy. T315I emerged in e.g. 45% of paients on dasatinib therapy. Overall probability of mutation emerging during therapy was 23%. 30 patients. Not all patients with T315I progressed interestingly. Patients with baseline murations had a higher probability of emerging mutations.

4.2. **[332] Prediction of Cytogenetic Response to Second Generation TKI Therapy in CML Chronic Phase Patients Who Have Failed Imatinib Therapy and Early Identification of Factors That Influence Survival.** Milojkovic *at al.* Second generation tyrosine kinase inhibitors (2G-TKI) have displaced allogeneic stem cell transplant as the preferred therapy for patients with CML in chronic phase (CP) who fail imatinib. However a significant proportion of patients still fail to respond to 2G-TKI and may benefit from alternative therapy (including stem cell transplant). We have performed univariate and multivariate analyses in our cohort of 80 patients treated with dasatinib (n=67) or nilotinib (n=13) while still in first CP after imatinib failure in order to identify those patients who will benefit most with these therapies. The median age was 50 years and 46% were male; 72 patients were resistant to imatinib (2 primary haematological resistance, 40 primary cytogenetic, 32 secondary cytogenetic and 25 developed secondary hematologic resistance) and 8 were intolerant. 20 had developed kinase domain mutations while on imatinib therapy. 31 and 29 patients received maximal doses of imatinib 600 and 800 mg per day respectively. The median follow up was 28.3 months (range 6-42). The 3-year cumulative incidence of CCyR was 52.6%. The multivariate analysis identified four pre-2G-TKI independent predictive factors for CCyR, namely low Sokal risk score at diagnosis, the best cytogenetic response obtained on imatinib, G-CSF requirement during imatinib therapy and time from detection of imatinib failure (as defined by European LeukemiaNet criteria) to onset of second 2G-TKI therapy. Using these factors we devised a scoring system that could be used to predict the probability of achieving CCyR on 2G-TKI therapy. The score was calculated by allocating one point when any one of the following four features was present: (1) intermediate or high Sokal risk group, (2) need of G-CSF support during imatinib therapy, (3) institution of 2G-TKI more than 18 months after imatinib failure, and (4) failure to achieve a cytogenetic response on imatinib (≥95% Ph-pos). The 3-year cumulative incidence of CCyR for patients with 0-1 points was 95.6%, with 2 points 50% and with 3-4 points 18.7% (p<0.0001, Figure 1). For the 80 patients the probability of 3-year survival was 89.6%. We performed a 3-month landmark analysis to study the

relationship between molecular response and subsequent outcome. The 44 patients with a BCR-ABL1/ABL ratio less than 15% at 3 months had a 3-year overall survival of 100% while the 36 patients with a ratio >15% had a survival of 77.4% (p=0.003, Figure 2). We performed a multivariate analysis including all relevant variables defined at the start of 2G-TKI and the 3-month transcript level. The 3-month transcript level was the only independent predictor for survival. Similarly we performed a 6-month landmark analysis where we explored the relationship between cytogenetic response and outcome. Patients who had achieved a MCyR (n=38) or a CCyR (n=32) had a significantly better survival than those with lower levels of cytogenetic response (100% vs. 79.2% (p=0.006) and 100% vs 82.6 (p=0.02)) respectively. We also performed a multivariate analysis including the variables defined at the initiation of therapy, the 3-month transcript levels and the cytogenetic response at 6 months. Interestingly the 3-month molecular response was the only independent variable predicting for survival. Similar results were found for progression-free survival (data not shown). We conclude that factors measureable before starting treatment with 2G-TKI may be valuable for predicting response; molecular responses at 3-months and cytogenetic responses at 6 months provide further information about the value of continuing treatment with 2G-TKI.

4.3. **[333] Molecular Response to First Line Imatinib Therapy Is Predictive for Long Term Event Free Survival in Patients with Chronic Phase Chronic Myelogenous Leukemia – An Interim Analysis of the Randomized German CML Study IV**. Müller *at al.* The introduction of imatinib has significantly changed prognosis of CML patients. Despite favourable hematologic and cytogenetic response (CyR) data, patients (pts) on first line imatinib therapy may relapse. Thus, studies have been conducted to improve initial therapy by dose escalation or combination with other drugs. CML Study IV was designed to compare imatinib in standard dose (400 mg/d) vs high dose (800 mg/d) vs combinations with low dose cytarabine or interferon alpha. We sought to evaluate the predictive impact of early molecular response for long term event free survival (EFS). 539 pts (59% m, median age 54 years, range 16-84) randomized to imatinib based therapies by December 2005 were investigated, the median follow up was 39 mo (range, 0-69). At baseline, multiplex PCR was applied to determine the dominating BCR-ABL transcript: b2a2 (n=204), b3a2 (n=247), b2a2 and b3a2 (n=80), e1a2 (n=2), e19a2 (n=4), b3a3 (n=1) and e8a2 (n=1). Quantitative PCR from 5,419 peripheral blood samples was performed using the LightCycler technology in two central labs. PCR data were aligned to the international scale (IS) by introduction of conversion factors (Hughes et al., BLOOD 2006). Cumulative molecular response of 539 pts at 3, 6, 12, 18, and 24 mo after randomization is summarized in the Table:

For analysis of prognostic impact, events were defined as (i) loss of complete hematologic response, (ii) loss of major CyR following loss of complete CyR, (iii) accelerated phase, (iv) blast crisis, and (v) death for any reason. Pts were censored at the time of allogeneic stem cell transplantation or switch to 2nd generation tyrosine kinase inhibitors because of imatinib intolerance or resistance. The minimum molecular response levels predictive for EFS were BCR-ABLIS of 10% after 6 mo (p=0.0029), 1% after 12 mo (p<0.0001), and 0.1% (major molecular response, MMR; p=0.0016) after 18 mo of imatinib based therapies. In order to investigate the reasons for unsatisfying responses BCR-ABL kinase domain mutations were assessed in 175 pts. 30 pts (17%) harbored 35 mutations affecting 18 different amino acids. In conclusion, prospective molecular surveillance of CML shows that early response predicts stable remissions on first line imatinib therapy. After 6 mo of treatment, PCR data start to be predictive for EFS. In pts with unsatisfactory response or molecular, cytogenetic and hematologic relapse, BCR-ABL mutations have been detected in only 17% of pts. Calculation of molecular response rates dependent on the various imatinib based therapies will be performed after stop of randomization which is expected by the end of 2009.

4.4. **[334] Reduction of BCR-ABL Transcript Levels at 6, 12, and 18 Months (mo) Correlates with Long-Term Outcomes on Imatinib (IM) at 72 Mo: An Analysis from the International Randomized Study of Interferon versus STI571 (IRIS) in Patients (pts) with Chronic Phase Chronic Myeloid Leukemia (CML-CP).** Hughes *at al.* Background: An exploratory endpoint of the IRIS trial was measurement of BCR-ABL transcripts over time and its correlation with long-term outcomes. BCR-ABL measured by polymerase chain reaction (PCR) was required per protocol only after achievement of a complete cytogenetic response (CCyR). However, preplanned substudies occurred at sites in Germany and Australia who conducted PCR measurements on pts at intervals from the start of treatment independent of cytogenetic response (CyR). Additionally, other IRIS investigators contributed non-protocol specified molecular assessments. This first entire PCR dataset from IRIS assesses the prognostic value of molecular response (MR) at specific time points. Methods: 553 pts were enrolled onto the IM arm of IRIS; of these, 476 pts with at least one PCR measurement form the basis for this analysis.

A major molecular response (MMR) is defined as the ratio of BCR-ABL/control gene (BAC) of ≤0.1%. Analyses were conducted at 6, 12 and 18 mo relating BAC percent reduction to event free survival (EFS), where events were defined as death during study treatment, loss of complete hematologic response, loss of Major CyR (MCyR), progression to accelerated phase (AP) or blast crisis (BC), or an increasing white blood cell count to > 20 x 109/L. Results: Among pts receiving first line IM for CML-CP, MMR was observed in 13% of samples available for study at 3 mo, 33% at 6 mo, 50% at 12 mo, 65% at 18 mo, 75% at 48 mo, 85% at 60 mo, and 86% at 72 mo. The degree of molecular response in pts who achieved CCyR is described in Table 1. This exploratory analysis demonstrates close correlation between CCyR and BAC ≤1% at 6 months and beyond.

At 6 mo, half of the pts with BAC >10% who also had a cytogenetic assessment at the same time had at least a partial cytogenetic response (PCyR) with an EFS of 91% at 72 mo, and 64% of these pts achieved MMR later. The other half of the pts with >10% BAC who did not have a PCyR at 6 mo had an EFS of 43%, and 31% later achieved MMR. A separate landmark analysis by CyR status alone showed EFS rates at 72 mo of 92% for pts in CCyR, 86% for pts in PCyR, 60% for Minor/Minimal CyR and 49% for No CyR. At 12 mo, pts with BAC ≤ 1% had excellent long term outcomes (72 month EFS of >90%, >95% without progression to AP/BC). Those pts with BAC > 1 - ≤ 10% (n = 36) had a 67% EFS, and 44% later achieved an MMR. These molecular analyses compare similarly to cytogenetic analyses alone (Baccarani et al; ASH 2006), with 60 mo EFS of 93% for pts in CCyR, 78% for pts in PCyR and 61% for pts without PCyR At 18 mo, pts with MMR could be statistically distinguished from pts with BAC >0.1 - ≤ 1%; EFS was 98% versus 89%, p=0.0137 (with 6 events in each group). The rate without AP/BC at 72 mo was not significantly different (with only 2 events in the >0.1 – ≤ 1% group). Baccarani et al (ASH 2006) reported an EFS at 60 mo of 96% for pts in CCyR, 80% for pts in PCyR and 69% for pts without PCyR. Table 2: Long-term outcomes (estimated rates at 72 mo) by MR levels at 6, 12 and 18 mo.

*P=.0137. None of the other comparisons between MMR and > 0.1 - ≤ 1% BAC were statistically significant. Conclusion: In pts on first-line IM, MMR rates increase over time, and in pts who achieved an MMR at any time point progression is rare. Achievement of a CCyR correlated well with BAC of ≤1% from 6 mo onwards. Exploratory molecular analyses show pts with BAC >10% at 6 mo have EFS rates distinguishable by their cytogenetic status. At 12 mo, pts with a BAC > 1% or without CCyR, fare more poorly than those with BAC ≤ 1% or those in CCyR. At 18 mo pts with BAC ≤ 1% have excellent long term outcomes, with the best outcomes seen in those with BAC ≤ 0.1%. Molecular and cytogenetic evaluations are recommended until at least CCyR is achieved, with molecular assessments measured indefinitely thereafter.

4.5. **[335] A Phase III, Randomized, Open-Label Study of 400 Mg Versus 800 Mg of Imatinib Mesylate (IM) in Patients (pts) with Newly Diagnosed, Previously Untreated Chronic Myeloid Leukemia in Chronic Phase (CML-CP) Using Molecular Endpoints: 1-Year Results of TOPS (Tyrosine Kinase Inhibitor Optimization and Selectivity) Study.** Cortes *at al.* Background: IM 400 mg/d is the standard of care for pts with newly diagnosed CML-CP. Previous reports suggest the rate of major molecular response (MMR), defined as BCR-ABL/control gene (BAC) ratio of ≤ 0.1% on the International Scale, predicts for a benefit in long-term outcomes. Phase 2 trials demonstrated that IM 800 mg/d as initial treatment of CML-CP decreases the time to MMR and increases the depth of molecular response (MR), and may therefore improve long-term outcomes. Methods: TOPS is a prospective, open-label, randomized (2:1) Phase 3 trial that compared IM 800 mg/d to 400 mg/d in CML-CP. Pts were stratified by Sokal risk score. The primary endpoint is MMR rate at 12 months (mo) and secondary endpoints include: rates of complete hematological response, complete cytogenetic response (CCyR), time to CCyR and MMR, progression to accelerated phase (AP) or blast crisis (BC), event-free survival (EFS), overall survival (OS), IM dose-intensity, pharmacokinetics, and safety. Rates were compared by Fisher's exact test and time to event outcomes by log-rank test. Results: 476 pts were enrolled (800 mg/d, n=319; 400 mg/d, n=157) at 103 sites in 19 countries between 6/05 and 12/06. Median age at diagnosis was 47 yrs, and 24% of pts had high Sokal risk score. Significantly more pts receiving IM 800 mg/d achieved MMR at 3 mo and 6 mo, but not at 12 mo when compared with 400 mg/d (Table 1). Time to MMR was faster in the 800 mg/d arm compared to 400 mg/d; P = .0038.

CCyR occurred faster in the 800 mg/d arm, indicated by a higher response rate at 6 mo (57% vs. 45%, P = .0146). At 12 mo rates of MMR and CCyR (ITT population) were higher for the 800 mg/d arm but were no longer significantly different (MMR 46% vs. 40%, P= .2035; CCyR 70% vs. 66%, P= .3470). In pts with high Sokal risk scores, rates of MMR at 12 mo were 41% and 26% (P = .1565) for the 800 mg/d and 400 mg/d arms, respectively. Exploratory analysis of MR at 3 mo and its correlation with achievement of

MMR at 12 mo follow. Of the pts in the 400 mg arm with BAC ratios >0.1- ≤ 1%, >1- ≤ 10% or > 10% at 3 mo, 83%, 46%, and 11% later achieved an MMR at 12 mo. In the 800 mg arm 73%, 45% and 21% of the pts with respective BAC ratios achieved an MMR at 12 mo. Based on the BAC ratio at 6 mo, the observed MMR rate at 12 months was 52%, 11%, and 0% in the 400 mg/d arm compared to 46%, 14%, and 18% in the 800 mg/d arm. In the first year of follow-up, 6 pts had documented progression to AP/BC during treatment: 3 (1.9%) in the 400 mg/d arm and 3 (0.9%) in the 800 mg/d arm. At 12 mo, 85% of pts in the 400 mg/d arm were receiving the randomized dose compared to 62% of pts in the 800 mg/d arm. Median DI was 400 mg/d in the 400 mg arm and 750 mg/d in the 800 mg arm. Dose interruptions > 5 days occurred more frequently in the 800 mg/d arm (67% vs 38%). Earlier achievement of MMR correlated with IM plasma trough level at 1 mo for the overall TOPS cohort; pts with IM concentrations < 1165 ng/mL (lowest quartile of the aggregate group) achieved MMR slower than those with concentrations ≥ 1165 ng/mL (p=.0149). The most common grade 3/4 nonhematologic toxicities were rash, diarrhea and myalgia occurring slightly more frequently in the 800 mg/d arm. Grade 3/4 hematologic toxicity occurred more frequently in pts receiving 800 mg/d. Conclusions: TOPS confirms the efficacy and safety of IM in newly-diagnosed CML-CP. MMR occurred earlier in pts treated with 800 mg/d and in patients with plasma IM level above the lowest quartile, reinforcing the utility of IM blood level testing to optimize treatment. DI of IM 800 mg/d was maintained and tolerability was good. Additional follow-up is required to evaluate the effect of dose and MR on long-term clinical outcomes.

NOTES FROM PRESENTATION

Levels of less than 1165ng/ml were associated with poorer outcome.

TOPS	**Overall**		**400mg**		**800 mg**		**Comment**
Parameter	No.	%	No.	%	No.	%	
No. patients total	476		157		319		
No. patients analysed							
Median age							
Prior mutations							
Median previous disease duration							
Median follow up (range)							
Median dose delivered							39% only could maintain 800mg.
CHR							
MCyR							
CCyR				66%		70%	At 12 months. **NS**
MMR				40%		46%	At 12 months. **NS.** Greatest difference seen in higher Sokal.
Median survival							
¾ anaemia							
¾ neutropenia				18		28	
¾ thrombopenia				10		18	
Lipase							
ALT							
Pleural effusion ¾							
QTc prolongation							
Rash ¾							Didn't seem to be significant differences.
Diarrhoea ¾							
Discontinued			12		31		

4.6. **[336] Identification of Novel Biomarkers for Prediction of Response to Tyrosine Kinase Inhibitors (TKIs) by Proteomic Profiling of Imatinib as Well as 2nd and 3rd Generation TKIs in Vitro.** Balabanov *et al.* The tyrosine kinase inhibitor (TKI) Imatinib (IM) represents the gold standard first-line treatment for patients with newly diagnosed chronic myeloid leukemia (CML). For patients developing resistance or intolerance to Imatinib, the 2nd generation TKIs Dasatinib (DASA) and Nilotinib (NILO) which are approved, and Bosutinib (BOSU) which is in clinical development, possess activity against almost all mutant forms of BCR-ABL which confer Imatinib-resistance, except the gatekeeper mutation, T315I. This latter mutation occurs in approximately 15% of clinically observed mutations in chronic phase

CML and confers resistance to all currently approved agents. Therefore compounds have been evaluated for activity against T315I mutant BCR-ABL, among which combined Aurora kinase and Abl inhibitors such as PHA-739358 (PHA) have been identified as showing some promise.In the current study, we used a classical proteomics approach to generate drug profiles of the TKIs (IM, DASA, NILO and PHA), in order to identify biomarkers that are either compound or drug group (i.e. 1st, 2nd or 3rd generation TKI) specific and could potentially be used as biomarkers for response prediction in vivo. For in vitro screening, we used murine Ba/F3 cells expressing wild-type (p210, wt) BCR-ABL or mutants, which are either low grade (M351T) or absolutely resistant (T315I) to Imatinib. Using 2D-gel electropheresis and mass spectrometry, we could identify a total of 68 individual protein spots which were differentially regulated in cells when treated with equieffective concentrations (IC50) of TKIs. Using in silico overlay of the different 2D-gels (Delta2D, Decodon GmbH Greifswald), 42, 38, 41 and 15 spots were found to be specifically differentially regulated in Ba/F3 cells expressing wt BCR-ABL under either IM, NILO, DASA and PHA, respectively. Interestingly, hierarchical cluster analysis based on these candidate proteins identified similar protein expression patterns for IM, NILO and DASA in comparison to PHA. Using genontology analysis (Panther software), the majority of the proteins belonged to the group of nucleic acid binding proteins (25%), cytoskeletal proteins (13%) and chaperones (12%). In contrast to the broad response of the different TKIs on wt Bcr-Abl cells, changes in protein expression patterns induced in cells carrying the M315T BCR-Abl mutation were substantially less pronounced (IM: 9, NILO: 12, DASA: 28, PHA: 17) with the strongest response seen in Dasatinib-treated cells, consistant with the compound being a powerful inhibitor of both wt and M351T BCR-ABL signaling. With the exception of the combined BCR-ABL and Aurora kinase inhibitor PHA (7 proteins), cells expressing T315I BCR-ABL exhibited no altered protein expression in response to treatment with either IM or the other 2nd generation TKIs. The protein expression patterns identified were used for systems biology network analysis using Metacore software (GeneGo), which enabled the elucidation of signaling pathways and identification of transcription factors involved in TKI response. Besides known regulators of BCR-ABL signaling, such as c-Myc and p53, we were able to identify novel TKI-dependent candidate proteins (e.g. eIF5a) and post-translational modifications (PTM) that, pending validation in primary patient material might effectively be used as biomarkers for response prediction in the near future.

5 Chronic myeloid leukemia - clinical trials 2 [abstracts 445 - 450]

5.1. **[445] Significance of Rising Levels of Minimal Residual Disease in Patients with Philadelphia Chromosome-Positive Chronic Myelogenous Leukemia (Ph+ CML) in Complete Cytogenetic Response (CGCR).** Kantarjian *et al.* Background. Patients with Ph+ CML receiving tyrosine kinase inhibitors (TKIs) are frequently monitored for response by quantitative polymerase chain reaction (QPCR) studies for minimal molecular disease. The clinical significance of rising levels of QPCR in CGCR is uncertain. Study Aims. To evaluate the relevance of increases of QPCR levels in patients with CML in CGCR on therapy. Study Group and Methods. Of 258 patients on imatinib therapy for newly diagnosed CML, 116 patients in durable CGCR on imatinib therapy for at least 18 months had significant QPCR increases (documented at least twice) as defined by literature reports. These were analyzed by the achievement of major molecular response (MMR; QPCR < 0.05%), and by the degree of QPCR increase. Results. The outcome of patients by disease status (still in MMR vs. loss of MMR vs. never in MMR) and by the QPCR level increase are shown in the Table. Only 13 of 116 patients (11%) with significant QPCR increases had CML progression; 11 of them were among 44 patients (25%) who either lost a MMR or never had a MMR, and had > 1 log increase of QPCR. The 5-year survival of all 116 patients was 92%, suggesting the minimal relevance of QPCR increases in patients in CGCR. Conclusion. Most patients with significant QPCR increases remain in CGCR. Patients who lose a MMR or never achieve a MMR, and have > 1 log increase of QPCR, should be monitored more closely, and may be evaluated for mutations of BCR-ABL kinase domain and considered for investigational therapeutic interventions. Allogeneic stem cell transplant should not be considered in view of the excellent survival.

5.2. **[446] Efficacy of Nilotinib (formerly AMN107) in Patients (Pts) with Newly Diagnosed, Previously Untreated Philadelphia Chromosome (Ph)-Positive Chronic Myelogenous Leukemia in Early Chronic Phase (CML-CP).** Cortes *et al*. Background: Nilotinib is an oral tyrosine kinase inhibitor with high selectivity towards Bcr-Abl and approximately 30-fold more potent than imatinib, and is effective in patients with CML after imatinib failure. We initiated a phase II study to evaluate the efficacy of nilotinib as 1st line therapy in pts with newly diagnosed CML-CP. Aims: To investigate the efficacy and safety of nilotinib as initial therapy for patients with CML-CP. Methods: The primary objective was to estimate the proportion of pts attaining major molecular response (MMR) at 12 months (mo). Pts with untreated CML-

CP (or with <1 months of therapy with imatinib) were eligible and received nilotinib 400mg twice daily. A cohort of patients with previously untreated CML in accelerated phase (AP) was also included. Results: Forty-nine pts have been treated for a median of 13 months (mo). The median age was 47 years (yrs) (range, 21 to 81); 69% are Sokal low risk. Eight (16%) had received imatinib for <1 months. Overall, 46/48 (96%) of evaluable CP pts achieved a complete cytogenetic response [CCyR]. The rate of CCyR at 3, 6 and 12 mo for pts in CP compares favorably to those observed in historical controls treated with imatinib 400mg or 800 mg daily:

MMR was observed in 45% at 6 mo and 52% at 12 mo. Two of 44 (5%) evaluable pts have achieved confirmed complete molecular response, and 3 others unconfirmed (ie, only achieved on their last assessment). Grade 3-4 hematologic toxicity (transient) included thrombocytopenia in 10%, neutropenia in 12%, and anemia in 2%. Grade 3-4 non-hematologic adverse events (regardless of causality) included elevation of bilirubin in 8% and lipase in 6%. 19 (36%) pts had transient treatment interruptions and 17 (32%) had dose reductions. The actual median dose is 800mg daily. Three pts have come off study: 1 pt's choice and 2 because of toxicity (1 liver, 1 pericardial effusion). One of them (liver toxicity) transformed to blast phase shortly after coming off study. Estimated 24 month EFS (event = loss of CHR, loss of MCyR, AP/BP, death, or off because of toxicity) is 95%. Conclusion: Nilotinib 400 mg twice daily induces a CCyR in nearly all patients as early as 3 months after the start of therapy with a favorable toxicity profile. Accrual is ongoing.

NOTES ON PRESENTATION

Primary endpoint is molecular response at 1 year. 400mg twice daily maximum 100 patients.

Nilotinib up front	**400mg bid**		**Comment**
Parameter	No.	%	
No. patients total	56, 53 in chronic phase		
No. patients analysed	53		
Median age			
Prior mutations			
Median previous disease duration			
Median follow up (range)	12/12		
Median dose delivered			
CHR		100	
MCyR			
CCyR	45/46	98	Best response achieved. 90% patients had achieved CCR at 3months
MMR	25/47	53	MMR is 40% at 12 months ?? 65% at 18 months
EFS		89%	At 24/12. All patients are still alive.
¾ anaemia		5	
¾ neutropenia		11	
¾ thrombopenia		9	
Lipase			
ALT			
Pleural effusion ¾			Elevated LFTs, rash and fatigue.
QTc prolongation			
Rash ¾			
Diarrhoea ¾			
Discontinuations			6 patients. 4 came off because of tox. I think 2 patients wnet to BC.

5.3. **[447] Imatinib (IM) Pharmacokinetic (PK) Exposure and Its Correlation with Clinical Outcome in Patients with Chronic-Phase Chronic Myeloid Leukemia (CML-CP) for 400 Mg and 800 Mg Daily Doses (Tyrosine Kinase Dose Optimization Study [TOPS]).** Guilhot *et al.* Background: Correlation between IM plasma level and clinical response has been previously reported [Larson et al.,

Picard et al.]. TOPS is an open-label, randomized, multicenter Phase III study investigating whether 800 mg of IM (400 mg twice daily) results in an improved efficacy compared with 400 mg daily IM in newly diagnosed, previously untreated CML-CP. This analysis reports IM trough plasma levels (Cmin) at both doses and their correlation with clinical response and safety parameters. Methods: IM PK trough samples were collected at time 0 (predose), and following 1, 6, 9, and 12 month treatment for both 400 mg/day (mg/d) and 800 mg/d arms. Plasma concentrations of IM and CGP74588 (major metabolite) were determined by a validated LC/MS/MS (liquid chromatography and tandem mass spectrometry) method. Correlation of IM exposure with clinical response (major molecular response [MMR] rates and time to first MMR) was assessed by grouping patients into quartiles based on their measured IM Cmin levels in month 1. For correlation with frequency of adverse events [AEs], an average Cmin (aCmin) over 12 months corrected for dose intensity was used for the analysis. Correlations were assessed for the entire evaluable population and for each dose group separately. Results: IM PK exposure was proportional to dose and stable over time. For the 400 mg/d dose (n=78-87), the median IM Cmin values at month 1, 6, 9, and 12 were 1190, 1060, 1210, and 1295 ng/mL, respectively; and for the 800 mg/d dose (n=148-167) the corresponding Cmin values for each month were 2720, 2340, 2170, and 2150 ng/mL, respectively. The intra-patient variability (CV%) was low and similar between the 400 mg/d and 800 mg/d doses, 25% and 27%, respectively. The inter-patient variability (CV%) was 38% for 400 mg/d and 58% for 800 mg/d. Despite this inter-patient variability there was a strong correlation between IM Cmin at month 1 (Table 1) and time to MMR or MMR at 3, 6, 9 and 12 months.

*No. of patients achieving MMR/total number of patients evaluable at each visit

Based on the evaluable population at month 12, patients with higher Cmin at month 1 (>1165 ng/mL, Q2-Q4) achieved MMR faster than patients with lower Cmin (<1165 ng/mL, Q1) (P=0.0149). The MMR rate at 12 months was 58% for Q2-Q4 group and 38% for Q1 group (P=0.0263). In the 400 mg/d group, the MMR rate at month 12 was 24% for patients with Cmin below 851 ng/mL (Q1 for 400 mg/d), compared to 56% for patients with Cmin above 851 ng/mL (Q2-Q4; P=0.0207). In the 800 mg/d arm, the overall MMR at 12 month was 50%, and no significant differences were observed between different Cmin quartiles, although it should be noted that the majority of patients (88%) at this high dose level achieved a Cmin above 1165 ng/mL as compared with 52% for the 400 mg/d group. Using aCmin over 12 months as a rough estimate of exposure including dose changes, a slightly higher incidence of all grade AEs for the most frequently reported AEs such as rash, diarrhea, fatigue, and all cause edema, was observed in patients in the highest quartile but no significant differences in the frequency of grade 3/4 AEs were observed. Conclusion: In TOPS, IM plasma trough level was proportional to dose and stable over time despite a high inter-patient variability which may have been attributable to dose changes. Patients with a IM Cmin in the lowest quartile showed a lower MMR rate at 12 months, whereas patients in the highest aCmin quartile showed a higher frequency of all grades of some AEs. The TOPS trial confirms previous observations that IM Cmin of approximately <1000 ng/mL are associated with poorer outcomes. Monitoring IM levels can provide an added benefit to CML patients on IM to achieve the best clinical outcomes.

5.4. **[448] Allogeneic Hematopoietic Stem Cell Transplantation (HSCT) in the Imatinib-Era: High Survival Rate Following Allogeneic HSCT after Imatinib Failure: Results of the German CML Study IV.** Saussele *et al.* Allogeneic HSCT remains an important option for patients with chronic myeloid leukemia (CML) who failed imatinib. Focus has been on second line tyrosine kinase inhibitors (TKI). Little is known on the outcome of HSCT for such patients. In July 2002, the German CML-Study Group activated a prospective randomized trial comparing different imatinib based strategies in chronic phase CML (CP). Elective early HSCT was considered for patients (pts) with EBMT score 0–1 for those with high disease risk, and after imatinib failure. By the end of July 2008, 1197 pts were randomized. In 80 (6,5 %) pts HSCT was documented. 52 pts were male (65%), 23 were high risk pts (28%) according to the Euro score. Median age at diagnosis was 37 years (yrs) (range 16-62), median time to HSCT was 12.6 months (mo, range 3.5-54 mo). EBMT score was 0-1 in 8 (10%), 2 in 10 (12%), 3-4 in 44 (55%) and ³ 5 in 18 pts (23%). Median follow-up after HSCT was 19 mo (range 0-59). Cumulative response rates prior to HSCT were 68% for complete hematologic response, 23% for complete cytogenetic responses, and 9% for major molecular responses. Based on the indication for HSCT three groups were defined: i) early HSCT (n= 19, 23%; low EBMT score (n=9), high risk pts (n=7), patient request (n=3); ii) HSCT after imatinib failure or intolerance in first CP (n=34, 43%) and iii) HSCT in second CP or higher, accelerated phase or blast crisis (n=27, 34%). 14 pts died, 10 deaths were transplantation related, 4 CML related. Two pts with a molecular relapse were successfully treated with donor lymphocyte infusion in combination with TKI. Overall survival rate at two yrs for group one was 87.8%, for group two 93.8%, and for group

three 49.5%. By EBMT score, survival rates were 100% for risk score 0-2, 82.2% for risk score 3-4, and 43.5% for risk score ³ 5. Data from this prospective controlled cohort study clearly show that HSCT remains an attractive and important rescue therapy for CML patients with imatinib failure or intolerance, particularly for those with a low EBMT risk score.

5.5. **[449] Dasatinib Efficacy in Patients with Chronic Myeloid Leukemia in Chronic Phase (CML-CP) and Pre-Existing BCR-ABL Mutations.** Müller *et al.* Dasatinib (SPRYCEL®) is an effective BCR-ABL inhibitor that is 325-fold more potent than imatinib and 16-fold more potent than nilotinib in vitro against unmutated BCR-ABL. Across a series of phase II and III trials, dasatinib has demonstrated durable efficacy in patients with CML following resistance, suboptimal response, or intolerance to imatinib. BCR-ABL mutations are an important cause of imatinib failure and suboptimal response. Here, the efficacy of dasatinib in patients with CML-CP who had baseline BCR-ABL mutations following imatinib treatment was analyzed using data from three trials (CA180-013, -017, and -034). Mutational assessment of the BCR-ABL kinase domain was performed using RT-PCR and direct sequencing of peripheral blood cell mRNA. Hematologic, cytogenetic, and molecular response rates were reported after ≥24 mos of follow-up. Duration of response, progression-free survival (PFS), and overall survival (OS; in 013/034) were calculated using Kaplan-Meier analysis, and rates were estimated at the 24-mo time point. Of 1,150 patients with CML-CP who received dasatinib, 1,043 had a baseline mutational assessment and were analyzed further. Of these, 402 patients (39%) had a BCR-ABL mutation, including 8% of 238 imatinib-intolerant and 48% of 805 imatinib-resistant patients. Excluding known polymorphisms, 64 different BCR-ABL mutations were detected affecting 49 amino acids, with G250 (n=61), M351 (n=54), M244 (n=46), F359 (n=42), H396 (n=37), Y253 (n=26), and E255 (n=25) most frequently affected. Dasatinib treatment in patients with or without a baseline BCR-ABL mutation, respectively, resulted in high rates of major cytogenetic response (MCyR; 56% vs 65%), complete cytogenetic response (CCyR; 44% vs 56%), major molecular response (MMR; 33% vs 45%); PFS (70% vs 83%), and OS (89% vs 94%) (Table). After 24 mos, CCyRs in patients with or without a BCR-ABL mutation had been maintained by 84% vs 85%, respectively, of those achieving this response. Among patients with mutations who received dasatinib 100 mg once daily, which has a more favorable clinical safety profile, efficacy and durability were similar (MCyR: 55%; CCyR: 41%; MMR: 36%; PFS: 73%; OS: 90%). In general, high response rates and durable responses were observed in patients with different mutation types, including highly imatinib-resistant mutations in amino acids L248, Y253, E255, F359, and H396. When responses were analyzed according to dasatinib cellular IC50 for individual BCR-ABL mutations, dasatinib efficacy was observed in 44 patients who had any of 5 imatinib-resistant mutations with a dasatinib cellular IC50 >3 nM (Q252H, E255K/V, V299L, and F317L, excluding T315I), including MCyR in 34%, CCyR in 25%, MMR in 18%, PFS in 48%, and OS in 81%. Among patients whose mutations had a dasatinib IC50 ≤3 nM (n=254) or unknown IC50 (n=83), responses and durability were comparable to patients with no BCR-ABL mutation. As expected, few patients with a T315I mutation (IC50 >200 nM; n=21) achieved a response. Among 70 patients with >1 mutation, a MCyR was achieved in 53% and a CCyR in 37%. Among patients with mutational analysis at last follow-up (n=162), 42 (26%) retained a BCR-ABL mutation (20 retained a mutation with IC50 >3 nM), 42 (26%) lost a mutation (5 lost a mutation with IC50 >3 nM), and 44 (27%) developed a new mutation (39 developed a mutation with IC50 >3 nM), with some patients counted in more than one category. Overall, this analysis demonstrates that dasatinib has broad efficacy against all BCR-ABL mutations except for T315I. For patients with BCR-ABL mutations, dasatinib treatment is associated with durable responses and favorable long-term outcomes.

	No BCR-ABL mutation	BCR-ABL mutation	BCR-ABL mutation treated with 100 mg QD	Analysis by dasatinib IC50		
				>3 nM (excl. T315I)	≤3 nM*	Unknown IC50**
Patients, n	641	402	49	44	254	83
CHR	93	90	90	82	94	96
MCyR	65	56	55	34	58	73
CCyR	56	44	41	25	47	54
MMR	45	33	36	18	34	43
Median time to MCyR, mos	2.8	2.9	2.8	5.7	2.9	2.8
Median time to	3.0	5.3	3.0	5.7	5.4	3.4

CCyR, mos						
24-mo PFS (95% CI), %	83 (79.8–86.5)	70 (65.3–75.2)	73 (60.1–86.3)	48 (31.2–64.7)	73 (66.6–78.9)	89 (82.3–96.3)
24-mo OS (95% CI), %	94 (91.4–95.7)	89 (85.1–92.1)	90 (81.2–98.3)	81 (68.8–93.8)	90 (85.8–94.2)	96 (91.2–100)

Some patients had >1 mutation. *Excluding patients with a concurrent mutation with dasatinib IC50 >3 nM. **Excluding patients with a concurrent mutation with known dasatinib IC50.

5.6. **[450] Dasatinib Time to and Durability of Major and Complete Cytogenetic Response (MCyR and CCyR) in Patients with Chronic Myeloid Leukemia in Chronic Phase (CML-CP).** Baccarani *et al.* Dasatinib (SPRYCEL®) is a potent BCR-ABL inhibitor that is 325-fold more potent than imatinib and 16-fold more potent than nilotinib in vitro against unmutated BCR-ABL. In this analysis, time to, duration, and rates of cytogenetic responses to dasatinib were determined using Kaplan-Meier analysis in patients recruited to phase II trials in imatinib-resistant or -intolerant CML-CP (START-C and -R), which have more than 2 years of follow-up. In both trials, patients received dasatinib at the previous dose of 70 mg twice daily (the approved dose in CML-CP is now 100 mg once daily following phase III dose optimization demonstrating improved tolerability). In START-C, imatinib-resistant and -intolerant patients were recruited, and separate analyses were performed for each group. In START-R, a randomized trial of dasatinib vs escalated-dose imatinib, only imatinib-resistant patients were recruited and patients from the dasatinib arm were analyzed prior to cross over. In all dasatinib trials, MCyRs and CCyRs were determined using conventional bone marrow cytogenetic assessment. Progression was defined as increasing white blood cell count, loss of complete hematologic response, loss of MCyR, transformation to accelerated or blast phase, or death. Among imatinib-resistant patients treated in START-C, a MCyR had been achieved at 3, 6, and 12 months by 29%, 40%, and 51%, and a CCyR had been achieved by 18%, 28%, and 39%, respectively (Table). At 24 months, MCyR and CCyR had been achieved by 55% and 44%, respectively. Among responding patients, median time to MCyR was 2.9 months and to CCyR was 5.5 months. In resistant patients who had achieved a MCyR, 94% and 84% had maintained this response 12 and 24 months after it had been initially achieved. For CCyR, 95% and 86% had maintained their response at 12 and 24 months, respectively. Progression-free survival (PFS) at 12 and 24 months for imatinib-resistant patients was 88% and 75%. In START-R, dasatinib response rates and durability were similar to those observed in the imatinib-resistant population of START-C, and median times to MCyR and CCyR were 2.8 and 4.1 months, respectively. Among imatinib-intolerant patients treated in START-C, MCyRs had been achieved at 3, 6, and 12 months by 62%, 74%, and 80%, and CCyRs by 44%, 65%, and 74%, respectively. Rates at 24 months had reached 82% for MCyR and 78% for CCyR. Median times to achieve MCyR and CCyR in the intolerant population were both 2.8 months. Among responding patients, 99% and 97% of intolerant patients were without loss of MCyR 12 and 24 months after responding, and 100% and 98% were without loss of CCyR, respectively. The 12- and 24-month PFS rates were 98% and 94%. In conclusion, dasatinib treatment results in high rates of durable MCyRs and CCyRs in patients with imatinib-resistant or -intolerant CML-CP, and responses are achieved rapidly in most patients.

	START-C			START-R
	Overall (N=387)	Resistant (n=288)	Intolerant (n=99)	Resistant (n=101)
CCyR achieved (%)				
3 months	25	18	44	22
6 months	37	28	65	29
9 months	44	34	73	35
12 months	48	39	74	37
18 months	51	42	78	43
24 months	53	44	78	44
Median time to CCyR (months)	3.2	5.5	2.8	4.1
Patients without loss of CCyR (%)				

12 months	97	95	100	97
24 months	90	86	98	94
PFS (%)				
12 months	91	88	98	91
24 months	80	75	94	86

6 Chronic myeloid leukemia - pathophysiology and TKIs [abstracts 569 – 576]

6.1. **[569] Structural Positioning of the SH2 Domain Is Critical for Bcr-Abl Kinase Activity, Signal Transduction and Oncogenic Transformation**. *Hantschel et al.* We have recently shown that the SH2 domain stimulates c-Abl catalytic activity and substrate phosphorylation. This effect is exerted directly through the establishment of a tight SH2-kinase domain interface in the active conformation of c-Abl (Filippakopoulos et al. (2008) Cell, in press, scheduled to be published on September 5, 2008). Mutations in the SH2 domain that presumably disrupt this SH2-kinase domain interface, such as the Ile164Glu mutation, result in severe impairment of Abl catalytic activity. Thus, correct positioning of the SH2 and kinase domain modules appears to be critical for efficient activation of cytoplasmic tyrosine kinases. Here, we present data showing that the same structural coupling of the SH2 and kinase domain is also a critical factor for full activation of the oncogenic fusion kinase Bcr-Abl. A single point mutation in the SH2 domain (Ile164Glu) led to a dramatic reduction in Bcr-Abl in vitro tyrosine kinase activity and Bcr-Abl autophosphorylation, both on the activation loop (pTyr-412) and the SH2-kinase domain linker (pTyr-245). This resulted in a strong decrease in global cellular tyrosine phosphorylation, as well as decreased phosphorylation of critical downstream mediators of Bcr-Abl signaling. Both wildtype Bcr-Abl, as well as the Bcr-Abl Ile164Glu mutant were able to confer factor independent growth to Ba/F3- and UT-7 cell lines, although to a different extent. Detailed data on the properties of the Ile164Glu mutation in vitro, in imatinib inhibition assays, transformation assays and mouse bone marrow transplant models will be presented. We propose that the structural positioning of the SH2 domain is a crucial factor for constitutive activity, signal transduction and transforming capacity of Bcr-Abl. Besides oligomerization via the N-terminal coiled-coiled domain and loss of the auto-inhibitory N-terminal myristoyl group, the proper positioning of the SH2 domain appears to be another critical factor that is required for constitutive activation of Bcr-Abl, which is the prerequisite for its ability to induce chronic myeloid leukemia (CML). Inhibitors of the SH2-kinase domain interface of Bcr-Abl may comprise alternative or additional points of pharmacological intervention for the treatment of imatinib-sensitive or -resistant CML or Ph+ acute lymphocytic leukemia.

6.2. [570] Distinct Gab2-Mediated Signaling Pathways Are Essential for Myeloid or Lymphoid Transformation and Leukemogenesis by BCR-ABL. *Mohi et al.* The *BCR-ABL* oncogene encodes an activated fusion tyrosine kinase that causes chronic myelogenous leukemia (CML) and B-lymphoid acute lymphoblastic leukemia (B-ALL) in humans. An autophosphorylation site at Tyr 177 of BCR-ABL recruits Grb2 via its SH2 domain, and is required for efficient induction of CML-like myeloproliferative disease by BCR-ABL in a mouse BM retroviral transduction/ transplantation model. We showed previously (Sattler et al., Cancer Cell 2002;1:479) that the scaffolding/adapter protein Gab2 is recruited to Y177 of BCR-ABL via a Grb2/Gab2 complex, and in vitro transformation of primary myeloid and lymphoid progenitors by BCR-ABL was impaired in bone marrow from mice with homozygous null mutations in the *Gab2* gene (*Gab2*$^{-/-}$ mice), coincident with decreased activation of the ERK and Akt signaling pathways. Here, we demonstrate an essential requirement for Gab2 in myeloid and lymphoid leukemogenesis by BCR-ABL. Whereas recipients of BCR-ABL-transduced Gab2$^{+/+}$ BM develop fatal CML-like myeloproliferative disease within 4 weeks of transplantation, recipients of BCR-ABL-transduced *Gab2*$^{-/-}$ BM fail to develop CML but succumb after a long latent period to T-cell acute lymphoblastic leukemia, phenocopying the disease induced by the BCR-ABL Y177F mutant. These results suggest that the Y177F and Gab2 mutations have an epistatic relationship, and that the critical transforming signals from Tyr177 of BCR-ABL are transmitted through Gab2. Co-expression of Gab2 with BCR-ABL in *Gab2*$^{-/-}$ BM restored efficient induction of CML-like leukemia, but mutants of Gab2 that lacked either the pleckstrin homology domain or Tyr binding sites for the SH2 domains of the downstream Gab2 effector molecules SHP2 or p85 PI3K failed to rescue myeloid leukemogenesis by BCR-ABL, although the mutant Gab2 proteins were expressed in circulating myeloid cells. Gab2 deficiency attenuated B-lymphoid transformation by BCR-ABL in vitro, and significantly prolonged the latency of B-ALL induced by BCR-ABL in mice. In contrast to CML, induction of B-ALL in Gab2$^{-/-}$ BM was rescued by either WT Gab2 or the Gab2 mutant defective in p85 binding. These results demonstrate that BCR-ABL absolutely requires signaling via Gab2 to both SHP2 and PI3K to cause CML, while a Gab2-SHP2 signaling pathway contributes to the pathogenesis of

BCR-ABL$^+$ B-ALL.

6.3. **[571] Lymphoid Blast Crisis Transformation and Development of Drug-Resistance in Chronic Myeloid Leukemia Are Driven by Aberrant Somatic Hypermutation.** Klemm et al. Chronic myeloid leukemia (CML) in chronic phase has a disease-free survival of 87% (DFS; 5 years) and long-term treatment with Imatinib is effective. In a large subgroup of patients with CML, however, the disease ultimately progresses into B lymphoid blast crisis (LBC) with only 6% DFS and resistance to Imatinib develops in virtually all cases. In most cases, acquired resistance to Imatinib can be attributed to somatic mutations within the BCR-ABL1 kinase domain. Whereas BCR-ABL1 kinase mutations are rare in chronic phase CML, such mutations are found in >80% of patients with B cell lineage LBC. Likewise, deletions of the ARF and INK4B genes are rare in chronic phase CML but found in ~50% of B cell lineage LBC. In a search for a B cell lineage-specific mutation mechanism responsible for BCR-ABL1 kinase mutations, we tested the hypothesis that aberrant activation of somatic hypermutation may give rise to drug-resistance and progression of chronic phase CML into LBC. Somatic hypermutation drives affinity maturation of immunoglobulins expressed by germinal center B cells and requires the cytidine deaminase AID. Expression of AID depends on PAX5, a transcription factor that determines B cell lineage commitment of hematopoietic progenitor cells. The dependence of AID expression on PAX5 limits somatic hypermutation to the B cell lineage. Consistent with aberrant activation of somatic hypermutation in B cell lineage LBC, we found both PAX5 and AID expression at the mRNA and protein level in B lymphoid but not myeloid subclones from patient-derived blast crisis CML. However, AID protein levels in LBC clones were 5-10-fold lower than in germinal center B cells. To confirm lineage-specific activation of AID-expression in BCR-ABL1 driven leukemia, we isolated bone marrow from Aid-GFP reporter transgenic mice and transformed the bone marrow cells with BCR-ABL1 under either myeloid (IL3, IL6, SCF) or B lymphoid (IL7) culture conditions. The Aid-GFP reporter drives GFP expression under control of upstream and downstream regulatory elements of the Aid locus (Crouch et al., 2007). BCR-ABL1-induced Aid-expression was only observed under B lymphoid culture conditions and was very heterogeneous among the leukemia cell population: Only about 5-10% of CD19$^+$ B lymphoid leukemia clones express Aid-GFP. In these cells, however, Aid mRNA levels are 240-fold higher than in Aid-GFP-negative cells and even 1.5-fold higher than in normal germinal center B cells. Consistent with these findings, we found aberrant somatic hypermutation of the IGHM, BCL6 and MYC loci as well as evidence of ongoing DNA single-strand breaks at the ARF and INK4B loci in B cell lineage LBC but not myeloid CML clones. Ectopic expression of AID in seven otherwise AID-negative CML cell lines cells leads to the acquisition of Imatinib-resistance and sequence analysis of the Imatinib-resistant clones revealed accumulation of mutations within the BCR-ABL1 kinase domain that cause Imatinib-resistance in patients (e.g. L248V, E225K, T315I). Aberrant expression of AID also caused Imatinib-resistance of CML cells in vivo: NOD/SCID mice were injected with CML cells that were either transduced with AID/GFP or GFP alone. Whereas more than the half of the mice injected with GFP$^+$ CML cells were still alive after 170 days, all mice in the AID/GFP$^+$ CML group died within 54 days after injection despite Imatinib-treatment. Forced expression of the B cell-specific transcription factor PAX5 in otherwise PAX5-negative CML cells resulted in a partial B lymphoid lineage conversion similar to LBC. Of note, ectopic expression of PAX5 also resulted in aberrant AID expression, subsequent acquisition of BCR-ABL1 kinase mutations and development of drug-resistance. We conclude that B cell-specific activation of PAX5/AID-induced aberrant somatic hypermutation provides a genetic basis for the strikingly different outcome of myeloid lineage CML as compared to LBC.

6.4. **[572] Functional p53 Is Required for Effective Telomerase Inhibition in BCR-ABL-Positive CML Cells in Vitro.** Brassat et al. Telomeres consist of repeat structures such as (TTAGGG)n in vertebrates and are localized at the end of chromosomes. Replication-dependent telomere shortening due to the end-replication problem can be counteracted by upregulation of an endogenous reverse transcriptase called telomerase. Increasing evidence suggests that critical telomere shortening results in genetic instability which may promote tumour evolution and telomerase activation during which critically short telomeres are stabilised and ongoing tumour growth is facilitated. In Chronic myeloid leukemia (CML) the high turnover of the malignant clone is driven by the oncogene BCR-ABL and leads to accelerated telomere shortening in chronic phase (CP) compared to telomere length in healthy individuals. Telomere shortening has been demonstrated to be correlated with disease stage, duration, prognosis and response to molecular targeted treatment. Despite of the accelerated telomere shortening observed, telomerase activity is increased in CP CML and further upregulated with progression of the disease to accelerated phase or blast crisis (AP/BC). To investigate the effect of telomerase inhibition on BCR-ABL-positive cells, we expressed a dominant-negative mutant of hTERT (vector pOS DNhTERT-IRES-GFP) in K562 cells. The cells were single sorted and clones in addition to bulk cultures were long

term expanded in vitro. The expression of the transgene DNhTERT was monitored by the expression of GFP and function of DNhTERT was analyzed by measurement of telomere length (by flow-FISH) and telomerase activity (TRAP assay). Evaluation of these parameters showed the following patterns of growth kinetics and telomere biology in individual clones: Two clones lost telomere repeats and were transiently delayed in growth kinetics but eventually escaped from crisis without loss of GFP expression (indicated by a re-increase in telomere length and growth rate, group A) Three other clones lost GFP expression after initial and significant telomere reduction indicating loss of the transgene (group B). Finally, telomere length and growth kinetics of two remaining clones and of the bulk culture cells remained unaffected by expression of DN-hTERT (group C). Of note, none of the clones analyzed either died or entered cell cycle arrest. Further analyses of one clone of group A revealed impaired DNA damage response indicated by two fold increase in number of γH2AX foci in comparison to control cells. Moreover, the expression pattern of genes involved in DNA repair was significantly altered (Dual chip®). Network analysis of the altered genes using MetaCore® software confirmed p53 as a key regulator in signaling of DNA damage in these cells. CML blast crisis cell lines such as K562 are typically negative for functional p53 and $p16^{INK4}$. Therefore, we went on and investigate if the presence of functional p53 is required for the induction of telomere-mediated apoptosis or senescence in BCR-ABL-positive cells. For this purpose, we restored p53 in telomerase-negative clones by using an inducible system (vector pBABE $p53ER^{tam}$) in two clones from group A and group B. Induction of p53 in cells with critically short telomeres (telomere length 4-5 kb) lead to immediate induction of apoptosis while vector control cells continued to escape from crisis. These results suggest that the success of strategies aimed at telomerase inhibition in CML is dependent on the presence of functional p53 in BCR-ABL-positive cells which argues in favour of applying these strategies preferentially in CP as opposed to BC.

6.5. **[573] Clonal Expansion of T/NK-Cells during Tyrosine Kinase Inhibitor Dasatinib Therapy.** Mustjoki et al. Tyrosine kinase inhibitors (TKIs) targeting the BCR-ABL fusion protein are an effective therapy for Philadelphia chromosome positive (Ph+) leukemia. Dasatinib, a 2nd generation pan-TKI, also inhibits wild-type kinases, which may result in unexpected clinical responses. Recent data suggest that dasatinib has a potent immunosuppressive effect on T- and NK-cells in vitro. In contrast, we have noticed a marked expansion of lymphocytes in blood in a subset of dasatinib patients, but its clinical significance and molecular mechanisms are unclear. In this multicenter study, 19 Ph+ leukemia patients with marked lymphoproliferation in blood while on dasatinib (9 CML CP, 2 CML AP, 2 CML BC, 6 Ph+ALL) were identified. Clonality, immunophenotype, and intracellular signaling was analyzed and related clinical information was collected. In addition, prevalence and prognostic significance of the phenomenon was retrospectively assessed in a Phase II clinical study on 46 Ph+ ALL patients. An abrupt lymphocytosis (peak count range 4-20x109/L) with large granular lymphocyte (LGL) morphology was observed after a median of 3 months from the start of therapy (range 1-8 months). In most patients, LGL lymphocytosis was long-lasting and continued throughout dasatinib therapy, albeit with marked fluctuations in absolute lymphocyte count (Fig. 1a). In all evaluable patients (n=4), lymphocyte counts normalized after drug discontinuation. All patients were previously exposed to imatinib without similar changes in lymphocyte count or morphology. By immunophenotyping, 14 patients had a cytotoxic T-cell and 5 patients a NK-cell phenotype. After initiation of dasatinib therapy, the CD4/CD8 ratio shifted, expression of activation antigens HLA-DR and CD57 increased, expression of homing antigen CD62L decreased and relative numbers of regulatory T-cells were significantly decreased ($p<0.001$). All patients with T-LGL phenotype had mono/oligoclonal rearrangements in TCR g/d genes. Quantitative allele-specific oligonucleotide PCR analysis from samples before and during dasatinib therapy indicated that LGL lymphocytosis results from expansion of a pre-existing terminal memory cell clone (CD8+CD62Llow). By using single-cell profiling of nodal phosphoproteins (ERK1/2, STAT1, STAT3, STAT5, STAT6) by multi-color FACS, we showed that during dasatinib therapy LGL patients were more responsive to cytokine or growth factor stimuli (IL-2, IL-4, IL-6, IFNs, GM-CSF), when compared to dasatinib patients without lymphocytosis or to healthy controls. This indicates alternative signaling pathway usage not inhibited by dasatinib and/or cytokine hypersensitivity. Dasatinib-related severe adverse effects were common in patients with LGL lymphocytosis. Pleural effusions and/or pulmonary infiltrates were seen in 13/19 patients, modest CMV reactivation in 9/19 patients, colitis in 11/19 patients and long lasting mild fever in 14/19 patients. Accumulation of pheno- and genotypically identical cytotoxic T-cells was also detected in pleural effusion and colitis biopsy samples. Adverse effects were temporally related to dasatinib therapy and emerged after the appearance of LGL lymphocytosis. All patients with relapsed poor prognosis leukemia (Ph+ ALL n=5, blastic phase CML n=2) achieved durable complete molecular responses (CMR) during dasatinib therapy, CMR still ongoing in 3 patients (follow-up 18+ months). In the confirmatory Phase II dasatinib study in Ph+ ALL (START-L), patients with lymphocytosis had superior survival compared to patients without lymphocytosis (Fig. 1b). In conclusion, we propose that by inhibiting immunoregulatory kinases,

dasatinib may induce a reversible state of aberrant immune reactivity in a distinct subset of patients and result in anti-leukemia and anti-host responses driven by cytotoxic T/NK LGL cells. Further studies pinpointing the mechanisms, such as the target antigens on the malignant cells and activation pathways are ongoing.

6.6. **[574] Gamma Delta T Cells (GDTCs) to Eliminate Minimal Residual Disease (MRD) in Chronic Myeloid Leukemia (CML) : A Pre-Clinical Model.** *Siegers et al.* GDTCs constitute a small subset of T-lymphocytes (<10%) involved in tumor immune surveillance and are an attractive option for adoptive immunotherapy. We are especially interested in their potential role in eliminating MRD in CML. As a first step, we determined whether or not GDTCs were part of the CML clone. 17-50 metaphases of GDTCs from each of 3 patients with documented Ph+ BCR-ABL+ CML were karyotyped and found to be Ph(-). Results were confirmed by fluorescence in situ hybridization analysis and quantitative RT-PCR for BCR-ABL. Using Miltenyi Biotec positive selection (MACS), we obtained high yield and purity of GDTCs isolated from healthy donor-derived peripheral blood mononuclear cells (PBMCs). 2.4 x 10^6 (± $8.6x10^5$) GDTCs (n=13) were isolated and expanded over 1000-fold in vitro using optimized cell culture conditions. Commercial sera were screened; the chosen serum supported up to 5 times greater expansion than autologous human serum, as had been used for CML-patient-derived GDTCs. 1mg/mL concanavalin A proved the optimal mitogen, with a 2524-fold GDTC expansion compared to PHA (1764-fold) and the anti-CD3 antibody, OKT3 (267-fold). In all cases, IL-2 and IL-4 (both 10 ng/mL) supplemented culture media; neither additional IL-7 (10 ng/mL) nor IL-15 (10 ng/mL), alone or combined, improved expansion of GDTCs. The initial post-isolation purity of 97.9% (± 0.9%) as assessed by flow cytometric (FACS) analysis was maintained for culture duration. We observed that expanded GDTCs obtained from both healthy donors and Ph(+) CML patients exhibited specific cytotoxic activity against Ph+ lines, K562 and EM-2. Expanded GDTCs from Ph(+) CML patients were co-incubated with K-562 at effector:target (E:T) ratios of 1:1 and 10:1 in triplicate for 3 and 24 hours. Percent cytotoxicity, defined as % reduction in colony-forming cells (CFC) after co-incubation of GDTCs compared to CFC generated by targets alone, was comparable at both ratios: ~50% at 3 hours, decreasing to 39% at 24 hours. GDTCs isolated from 9 healthy donors were expanded in vitro and co-incubated with K562 or EM-2 at E:T of 20:1. Apoptotic cells were identified by a FACS cytotoxicity assay based on Annexin V staining. After 4 hours co-culture, K562 were 27% ± 2% and EM-2 45% ± 3% apoptotic. K562 cultured alone did not show increased Annexin V staining. Comparable results were obtained by standard chromium release assay. Simultaneous incubation of autologous PBMC with GDTCs did not yield significant PBMC Annexin V staining, confirming selective cytotoxicity of GDTCs against leukemic cells. Results were verified by incubating GDTCs from 3 healthy donors with autologous PBMCs mixed with 1.0%, 0.5%, or 0.1% K562eGFP+ cells at 20:1 E:T for 0 or 4 hours. Co-incubation with GDTCs resulted in significant and similar reduction in K562eGFP+ colonies at all ratios (96.5%, 96% and 97%, respectively). GFP-negative colony numbers were maintained after 4 hours co-incubation with GDTCs indicating specificity against K562eGFP. Similarly, GDTC co-incubation with autologous PBMCs generated 105% BFU-E and 117% CFU-GM colonies, comparing CFC generated from t=4 to t=0 hours co-culture, indicating lack of cytotoxicity against autologous committed hematopoietic progenitors. We confirmed cytotoxic efficacy of GDTCs in vitro by RT-PCR. 16 hours co-incubation of GDTCs with EM-2 resulted in a 2 log reduction in BCR-ABL transcripts. We have established a xenogeneic mouse model of CML with fluorescent and bioluminescent leukemic cells (K562eGFPluc) that we visualize via in vivo imaging system (IVIS®) technology. Both K562eGFPluc and CFSE-labelled GDTCs engraft in NODscidIL2Rg-/- mice. We are investigating cytotoxicity of GDTCs against CML MRD in this pre-clinical animal model.

6.7. **[575] Clonal Hematopoiesis in Philadelphia Chromosome-Negative Bone Marrow Cells of Chronic Myeloid Leukemia Patients Receiving Tyrosine Kinase Inhibitors.** *Paquette et al.* Patients with chronic myeloid leukemia (CML) are experiencing prolonged survival due to successful therapy with tyrosine kinase inhibitors. However, some CML patients who have achieved longstanding remissions with these agents harbor clonal cytogenetic abnormalities in their Philadelphia chromosome negative (Ph-) bone marrow cells. Because CML patients in remission often have peripheral blood count abnormalities, including cytopenias, we investigated whether these patients may have developed myelodysplastic syndrome (MDS) within the Ph- cell population. Bone marrow samples from 26 CML patients who had achieved a major cytogenetic remission (MCyR) with tyrosine kinase inhibitor therapy between 2 and 15 years after diagnosis were evaluated; 6 patients had advanced disease prior to their last therapy, 20 were in chronic phase. At the time of evaluation, 2 of the patients were receiving imatinib, 23 dasatinib, and 1 PHA739358. At least one peripheral blood lineage was abnormal in 21 patients, of whom 7 had pancytopenia. Routine metaphase cytogenetics (MC) revealed a persistent clonal chromosomal abnormality in ≥10% of the Ph- metaphases in 5 patients (+8 in 2, -7 in 2, and 20q- in 1). We

hypothesized that clonal hematopoiesis might exist in additional patients and applied single nucleotide array (SNP-A) based karyotyping and X-linked human androgen receptor (HUMARA) clonality assay to further delineate the nature of the hematopoietic defect in these patients. HUMARA was performed on bone marrow samples and germ-line DNA from peripheral blood T lymphocytes of the female patients. Clonality, as assessed by skewing of X-chromosome inactivation in bone marrow cells compared to germline control cells, could not be demonstrated in the12 female patients. SNP-A karyptyping using 250K Affymetrix SNP array confirmed the known cytogenetic abnormalities. Several microdeletions were found, but comparison with purified T lymphocytes demonstrated that these "lesions" represented germ line-encoded copy number variants. However, SNP-A karyotyping revealed the presence of uniparental disomy (UPD) involving chromosome 17(p12-pter) in bone marrow, but not germ line cells, from one male patient with normal karyotype by routine MC. In the context of secondary AML, del17p or UPD17 have been observed always in the presence of del7/q and 5q and were associated with poor prognosis. However, in our patient UPD17 occurred as a sole defect. Because in our studies in AML, UPD of chromosome 17p was found in association with p53 mutations, genomic sequencing of this gene was performed. A 5 bp deletion destroying the splice acceptor region of exon 6 was identified in bone marrow cells from this patient. Alternative splicing leading to loss of exon 6 was predicted to result in a frame shift and premature introduction of a stop codon. These methods revealed clonal hematopoiesis in the Ph- bone marrow cells of 6/26 patients with longstanding CML in remission from tyrosine kinase inhibitors and persistent peripheral blood abnormalities. The approaches used here probably underestimate the frequency of this condition, as oligoclonal populations may be present in numbers below the limit of assay sensitivity. The Ph- clonal bone marrow populations have cytogenetic and molecular features in common with MDS. After a median follow up of two years, one patient with monosomy 7 developed acute myeloid leukemia, but longer follow up will be required to determine the natural history of the Ph- clonal disorders.

6.8. **[576] Switch Pocket Inhibitors of the ABL Tyrosine Kinase: Distinct Kinome Inhibition Profiles and in Vivo Efficacy in Mouse Models of CML and B-Lymphoblastic Leukemia Induced by BCR-ABL T315I.** *Etten et al.* Acquired resistance to the ABL tyrosine kinase inhibitor (TKI) imatinib is a significant clinical problem for patients with chronic myeloid leukemia (CML), where a major resistance mechanism is mutations in the ABL kinase domain that render the BCR-ABL enzyme insensitive to the drug. The gatekeeper mutation Thr 315 Ile (T315I) arises at high frequency in patients relapsing after sequential therapy with second-line TKIs such as dasatinib or nilotinib (Shah et al., J Clin Invest 2007;117:2562), and confers pan-resistance to all ATP-competitive TKIs. Using structure-based rational drug design, we have developed a novel chemical class of compounds that bind to five distinct structural pockets that the ABL kinase uses to switch between the inactive and active conformations. Diversity in these "switch pockets" between kinases can be exploited to fine-tune the selectivity of an inhibitor against the rest of the kinome. Two development candidate ABL inhibitors have emerged from this effort: DCC-2036 and DCC-2157. Both compounds potently (IC_{50} = 0.8-4.0 nM) inhibit purified ABL in the switch-off (unphosphorylated) and switch-on (phosphorylated) states via a non-ATP-competitive mechanism, and avoid steric clash with Ile 315. Both drugs also impair proliferation and induce apoptosis of Ba/F3 cells expressing a wide variety of BCR-ABL TKI-resistant mutants, including T315I, many P-loop mutants, and the dasatinib-resistant mutant F317L. DCC-2036 is highly selective for ABL, FLT3, TIE2, and Src family kinases, and when dosed at 100 mg/kg/day by oral gavage, significantly prolonged the survival of mice with CML-like myeloproliferative disease induced by retroviral expression of BCR-ABL WT and T315I in bone marrow. Genetic studies in mice (Hu et al., Nat Genet 2004;36:453) suggest that the Src inhibitory activity of DCC-2036 would be beneficial against Ph^+ B-cell acute lymphoblastic leukemia (B-ALL), and indeed DCC-2036 was superior to dasatinib (Hu et al., PNAS 2006;103:16870) in prolonging survival of mice with B-ALL induced by BCR-ABL T315I. By contrast, DCC-2157 is selective for ABL, KIT, and PDGFRA/B kinases, and pharmacodynamic studies demonstrated prolonged (>18 hour) inhibition of phospho-Stat5 in $BCR\text{-}ABL^+$ leukemic cells following a single oral 100 mg/kg dose. Treatment of mice with CML-like disease induced by BCR-ABL T315I with DCC-2157 at 50 mg/kg every other day induced virtually complete suppression of leukemia and extensive prolongation of survival. These results demonstrate that switch pocket drug design technology yields ABL TKIs with distinct pharmacologic profiles that are promising new weapons for the treatment of de novo and drug-resistant Ph^+ leukemias.

7 Drug resistance and new drugs in chronic myeloid leukaemia [abstracts 721 - 726]

7.1. **[721] P-Glycoprotein (Pgp) - Dependent Drug Resistance to Imatinib at CML-BC Is Exclusively Developed in Aggressive Minor Blast Subpopulation (MS) and Can Be Reversed by Pgp Modulators.** *Galski et al.* CML is considered as a model of multi step-developing malignancies. Although very effective in chronic phase CML, imatinib mesylate (IM) and second generation TK inhibitors

treatment are usually less effective in advanced CML (accelerated and blast crisis phases) since drug-resistant clones inevitably shortly emerge. In a recent study (Simanovsky M et al, Differentiation. 2008 Apr 29 [Epub ahead of print]), we have demonstrated that at blast crisis CML (CML-BC), blood circulating blasts of the same CML clone are heterogeneous, containing a small cell-fraction (1-3%) of blasts that are significantly more aggressive than the major malignant population. Briefly, we found that these minor subsets (MS) of blasts (both from patients and human CML-BC cell lines) have a typical highly repopulating ability, increased clonogenicity, and over expression of *BCR-ABL* and few other cancer-related genes. To evaluate whether the MS blasts also exhibit differential drug resistance mechanisms toward IM, we compared the two blast subsets for the level of resistance to IM in relation to expression of a functional Pgp, an ABC transporter that is the product of the ABCB1 (MDR1) gene. In the current study, we found that the MDR1 gene (but not several other ABC transporter genes) is significantly (5-7 fold) upregulated in the MS blasts, relatively to the major population. Moreover, FACS and Western analyses revealed that while Pgp could not be detected on the cell surface of the major blast subsets, Pgp is exclusively highly expressed in the MS blasts. Moreover, functional Pgp assays in the MS blasts (efflux, dose-dependent competitions, and UIC2 Pgp-specific shift assays) indicated unequivocally that IM is a substrate for Pgp. While IM efficiently inhibited the proliferation of the major blasts in dose-dependent manner, the proliferation rate of the MS blasts was essentially not affected. Furthermore, the anti-proliferative effect of IM on the MS blasts could be restored by addition of the Pgp inhibitor, R-verapamil, in dose-dependent manner. While relatively long, gradual selection in culture of the major CML-BC subsets resulted in some Pgp-independent IM-resistant clones, Pgp activity levels were shortly further elevated (by 1-order magnitude) in the MS blasts. Interestingly, FACS analyses, using different monoclonal antibodies that bind specifically to different known extra cellular epitopes of Pgp, indicated differential antibodies-epitopes binding ratios after IM selection. These stoichiometric changes suggest a topological folding shift of Pgp between its moderate to high activity (proposed model, Figure 1). In conclusion, the existence of a minor "pool" of CML blasts of both greater clonogenicity and high expression and activity levels of Pgp, apparently signify clonal evolution toward both increased malignancy and lower therapeutic sensitivity. Moreover, as both IM and Dasatinb are transported by Pgp, this study suggests that their combination therapy with Pgp-modulateors might also be clinically effective in targeting this aggressive blast population.

7.2. **[722] Philadelphia-Positive Acute Lymphoblastic Leukemia Patients Already Harbor Bcr-Abl Kinase Domain Mutations at Low Levels at the Time of Diagnosis - a Report by the GIMEMA ALL Working Party.** *Colarossi et al.* In Philadelphia-positive (Ph+) acute lymphoblastic leukemia (ALL) patients (pts) treated with tyrosine kinase inhibitors (TKIs), responses are generally short-lived and relapse is most often accompanied by selection of point mutations in the Bcr-Abl kinase domain (KD). In order to assess whether mutations are already present at the time of diagnosis, we analyzed RNA samples from 13 pts with newly diagnosed Ph+ ALL enrolled on the GIMEMA LAL1205 protocol – designed to assess activity, safety and tolerability of dasatinib monotherapy as first-line treatment for adult Ph+ ALL. The pts investigated (males/ females: 6/7; median age: 56 years, range 30-73) included 6 pts who subsequently relapsed with dasatinib-resistant mutations, 2 pts who relapsed without evidence of mutations as assessed by direct sequencing and 5 pts in persistent remission. Screening for low level mutations was performed by cloning the entire Bcr-Abl KD (amino acid 206 through 524) in a bacterial vector and sequencing 150 to 200 independent clones for each pt. All pts, irrespective of their subsequent response to dasatinib, had evidence of aberrant KD sequences due to point mutations (13/13 pts), small insertions (2/13 pts) or small deletions (1/13 pts). Two to five point mutations were detectable for each pt. In some cases, multiple mutations could be found to co-exist in the same cloned fragment. A total of 45 different point mutations (including 18 silent mutations, 4 nonsense mutations and 23 missense mutations) were observed. Base substitutions were scattered all over the KD and there were no mutation hotspots. Thirty-nine out of 45 (87%) mutations were transitions: G>A (n=14), A>G (n=10), C>T (n=9), T>C (n=6). Such a high prevalence of transitions (which normally occur 1.4 times more frequently than transversions) suggests that a specific mechanism generating mutations is predominant. Activation-induced cytidine deaminase (AID) has been recently shown to be aberrantly expressed in a proportion of Ph+ ALLs and has been implicated in the development of at least some Bcr-Abl mutations; correlation with AID expression will be presented. Mutations were detected in no more than three independent clones, suggesting that they were present at low levels. Mutations detected in less than two clones were considered only if independently validated by a specifically designed ASO-PCR assay run on the original cDNA sample. The majority of point mutations detected have never been reported in association with TKI resistance. However, three pts were found to harbor known imatinib- or dasatinib-resistant mutations, including two T315I mutations that were later on selected and led to relapse. This supports the theory that mutations randomly arise as a consequence of a high genetic instability and very few of them will confer a

growth advantage under the selective pressure of TKIs. Six samples from newly diagnosed chronic phase CML (CP-CML) pts were screened for comparison with the same cloning approach, including 2 pts who later progressed with evidence of KD mutations after 3 and 6 months from the start of imatinib, respectively, and 4 pts who achieved a stable molecular response on imatinib. Both pts who experienced early relapse on imatinib had low level mutations detectable at the time of diagnosis, whereas none of the clones from CP-CML imatinib responders had evidence of KD mutations. The results herein presented support the concept that Ph+ ALL cells have a particularly high genomic instability that allows early escape from TKI inhibitor therapy. Only a fraction of CML cases share this feature. Our data highlight the importance of understanding the mechanism(s) responsible for this 'mutator' phenotype, as well as how to interfere therapeutically with it. Study supported by AIL, AIRC, PRIN and FIRB funding.

7.3. **[723] Enhancing the Functional Activity of the OCT-1 Influx Pump May Overcome the Negative Impact of Low OCT-1 Activity in Imatinib Treated CML Patients**. *White et al.* The human organic cation transporter-1 (hOCT-1) is the major active influx protein responsible for the transport of imatinib into blood cells [1,2]. The functional activity of the OCT-1 protein is defined as the intracellular uptake and retention (IUR) of 14-C labelled imatinib into patient pre therapy mononuclear blood cells over a two hour period, which is inhibited by OCT-1 inhibitors such as prazosin or procainamide. The level of OCT-1 activity is a key determinant of the interpatient variation observed in intrinsic sensitivity to imatinib induced kinase inhibition ($IC50^{imatinib}$ [3]). We have previously demonstrated that a significantly greater proportion of de-novo CML patients with high functional activity of OCT-1, achieve a major molecular response (MMR: 3 log reduction in BCR-ABL mRNA from standardised baseline) when treated with imatinib, than patients with low OCT-1 Activity [4]. We have also identified a link between dose and OCT-1 Activity, demonstrating that the negative impact of low OCT-1 Activity could be overcome to a variable extent by imatinib dose increase. However, not all patients can dose increase, largely because of tolerability issues. While the transport of second-generation ABL-kinase inhibitors (nilotinib and dasatinib[5]) is not OCT-1 mediated, the long term effect of these drugs is not yet known. Hence, we sought to identify strategies to increase OCT-1-mediated imatinib uptake. We queried the drug gene expression signatures in version 1 of the Connectivity Map (CMAP; Lamb J, Nat. Rev. Cancer 7; 54-60, 2007: http://www.broad.mit.edu/cmap) with 3 transporters including OCT-1. This identified the Rho kinase inhibitor fasudil and COX-2 inhibitor / celecoxib analogue LM1685 as potential up-regulators of OCT-1 mRNA. The impact of these drugs on OCT-1 mRNA expression and $IC50^{imatinib}$ (fasudil alone to date) has been analysed in two bcr-abl positive cell lines (K562 and KU812). The effect of these two candidate OCT-1 enhancers on OCT-1-mediated imatinib uptake was also assessed in 10 newly diagnosed chronic phase CML patients, previously demonstrated to have low OCT-1 Activity (4 with no demonstrable OCT-1 Activity), using the IUR assay. These data demonstrate a statistically significant increase in OCT-1 Activity with LM1685, and show a strong trend towards significance with fasudil. Importantly, we show that patients with no demonstrable OCT-1 Activity (0ng/200,000 cells) have detectable Activity in the presence of both fasudil (Range 1.5 to 2ng/200,000 cells) and LM1685 (Range 1.5 to 4.5 ng/200,000 cells). We have previously demonstrated that patients with no detectable OCT-1 Activity universally fail to achieve imatinib therapeutic response milestones (imatinib failure), whereas 54% of patients with low, but detectable OCT-1 Activity achieve these milestones[4]. The ability to enhance the functional activity of the OCT-1 protein may therefore be of significant clinical relevance in this group. In addition we demonstrate an increase in imatinib IUR which, along with the increase in OCT-1 Activity, is likely associated with increased OCT-1 mRNA levels. Significantly, in the two CML cell lines tested we show a marked reduction in the $IC50^{imatinib}$ indicating that the observed increase in IUR and OCT-1 Activity translates to an increase in the kinase inhibitory activity of imatinib. Preliminary analysis in one patient analysed to date also indicates a reduction in IC50 from 0.48 to 0.35μM in the presence of fasudil. In the clinical scenario the use of such OCT-1 enhancers may improve the response of some imatinib treated patients to both standard and increased dose imatinib. Importantly, these findings validate the use of resources such as C-MAP to identify candidate drugs that may mediate desired changes in the levels of key proteins resulting in improved response to therapy.

7.4. **[724] Combined Effects of Novel Heat Shock Protein 90 Inhibitor NVP-AUY922 and Nilotinib against Mutant Forms of BCR-ABL.** *Tauchi et al.* NVP-AUY922 (Novartis) is a novel 4,5-diaryloxazole ATP-binding site heat shock protein 90 (HSP90) inhibitor, which has been shown to inhibit the chaperone function of HSP90 and deplete the levels of HSP90 client protein. Combining AUY922 with ABL kinase inhibitors may provide several advantages, such as enhanced efficacy and reducing the potential emergence of new resistant mutations. Treatment with AUY922 has been shown to exert greater potency against BCR-ABL mutants compared with wild type (wt) BCR-ABL. In the present study, we investigated the combined effects of AUY922 and Nilotinib on mutant forms of BCR-ABL-expressing cells. Co-

treatment with AUY922 and Nilotinib resulted in significantly more inhibition of growth than treatment with either agent alone in BaF3 cells expressing wt-BCR-ABL and BCR-ABL mutants (M244V, G250E, Q252H, Y253F, E255K, T315A, T315I, F317L, F317V, M351T, H396P). The observed data from the isobologram indicated the synergistic effect of simultaneous exposure to AUY922 and Nilotinib even in BaF3 cells expressing T315I. In contrast, we did not observe enhanced effects of AUY922 and imatinib in T315I BCR-ABL-expressing cells. Co-treatment with 500nM of Nilotinib significantly increased AUY922-induced apoptosis in BaF3 cells expressing T315I. Combined treatment with AUY922 and Nilotinib in BaF3 T315I also associated with more PARP cleavage, resulting from increased activation of caspase-3 and -9 during apoptosis. Following co-treatment of BaF3 T315I with AUY922 and Nilotinib caused more attenuation of phospho-T315I BCR-ABL levels and the downstream signal transducer, including phosho-CrkL, phospho-Stat5, and phospho-Akt. These results demonstrate that Nilotinib appears to inhibit T315I BCR-ABL kinase activity in BCR-ABL structurally compromised by loss of HSP90 chaperone activity. To assess the in vivo efficacy of AUY922 and Nilotinib, athymic nude mice were injected i.v. with mixture of BaF3 cells expressing wild type BCR-ABL and mutant forms of BCR-ABL(M244V, G250E, Q252H, Y253F, E255K, T315A, T315I, F317L, F317V, M351T, H396P). 24 hrs after injection, the mice were divided four groups (5 mice per group), with each group receiving either vehicle, AUY922 (50mg/kg; i.p. two times per week), Nilotinib (30mg/kg; p.o. once every day), AUY922 (50mg/kg; i.p. two times per week) + Nilotinib (30mg/kg; p.o. once every day). Animals treated with either vehicle or Nilotinib-alone died of a condition resembling acute leukemia by 28 days; animals treated with AUY922 alone survived more than 40 days, and those treated with the combination of AUY922 + Nilotinib survived more than 60 days. Histopathologic analysis of vehicle or Nilotinib-alone treated mice revealed infiltration of the spleen and bone marrow with leukemic blasts. In contrast, histopathologic analysis of organs from AUY922 plus Nilotinib-treated mice demonstrated normal tissue architecture and no evidence of residual leukemia. Taken together, these preclinical studies show that the combination of AUY922 and Nilotinib exhibits a desirable therapeutic index that can reduce the in vivo growth of mutant forms of BCR-ABL-expressing cells, including T315I, in an efficacious manner.

7.5. **[725] Beyond the Gatekeeper: Imatinib- and Dasatinib-Resistant BCR-ABL/F317 Mutations Confer Cross-Resistance to VX-680 but Are Sensitive to a Structural Derivative of VX-680.** *Kasap et al.* The management of chronic phase CML has been revolutionized by selective ABL tyrosine kinase inhibitor (TKI) therapy. Despite the effectiveness of these targeted agents, long-term control of blast phase CML and Ph+ ALL has been elusive, where the majority of patients relapse within 6-12 months. For blast phase CML and Ph+ ALL, two TKIs are currently approved: imatinib and dasatinib. While head-to-head comparisons of these agents have not been performed, it is generally believed that dasatinib is the more active agent for these phases of disease. In most cases, loss of response to these agents is driven by BCR-ABL kinase domain mutations. While more than 70 mutations have been associated with clinical resistance to imatinib, dasatinib appears vulnerable primarily to five mutations: V299L, T315A, T315I, F317I, and F317L. Of these, T315I and F317L are cross-resistant to imatinib. For the achievement of long-term remissions in blast phase CML and Ph+ ALL, a combination of TKIs that can collectively suppress all resistant BCR-ABL kinase domain mutations holds therapeutic promise. The BCR-ABL/T315I mutation, which confers a high degree of resistance to all approved BCR-ABL TKIs, has been referred to as a “molecular gatekeeper”, as it restricts access to a deeper hydrophobic pocket within the ABL kinase domain and makes an important stabilizing H-bond with imatinib, dasatinib and nilotinib. The Aurora kinase inhibitor VX-680 was the first compound to have activity against BCR-ABL/T315I in vitro, as well as clinically. To determine the promise of a kinase inhibitor combination of dasatinib and VX-680, we assessed the activity of VX-680 against the five dasatinib-resistant mutations using a cell-based flow cytometric assay of BCR-ABL kinase activity. While three mutants are sensitive, mutations at F317 demonstrated a high degree of resistance. We tested a number of other Aurora kinase inhibitors of different chemotypes and found that each of these had similar difficulty at inhibiting the kinase activity of BCR-ABL/F317 mutants. Based upon the co-crystal structure of VX-680 complexed with ABL, we have performed structure-activity relationship studies of 12 VX-680 scaffold derivatives, and have successfully identified structural modifications that increase kinase inhibitory activity against F317 mutants. Moreover, one of these derivatives increases the selectivity for ABL relative to Aurora kinases, which may help reduce the likelihood of suppressing normal hematopoiesis, a dose-limiting toxicity of Aurora kinase inhibitors that may substantially limit their effectiveness for the management of hematologic malignancies such as blast phase CML and Ph+ ALL. Lastly, we have performed structural studies of ABL/F317 mutants complexed with select VX-680 derivatives in an effort to understand how F317 mutations confer resistance to a broad range of ABL and Aurora kinase inhibitors. Interestingly, a recent study reported the successful selection of Aurora kinase inhibitor-resistant clones derived from a human colon cancer cell line (Girdler et al, 2008). While no resistance-conferring mutations were isolated at L154, the Aurora

kinase gatekeeper residue, mutations were detected at Y156 in Aurora B, which corresponds to F317 in ABL. Aurora B Y156 mutations were found to confer resistance to a number of Aurora kinase inhibitors, including VX-680. As Aurora kinase inhibitors are being studied in a variety of non-hematologic malignancies, there is an increasing need to understand and overcome the mechanisms whereby mutations at this residue confer resistance to these agents. It is hoped that our studies will lead not only to the development of an effective adjunctive kinase inhibitor for the treatment of blast phase CML and Ph+ ALL, but will also shed light on the growing problem of resistance conferred by mutations at residues that correspond to BCR-ABL/F317 in other kinases.

7.6. **[726] Complete Suppression of in Vitro Resistance by AP24534, a Pan-BCR-ABL Inhibitor.** *O'Hare et al.* The BCR-ABL inhibitor imatinib is front-line therapy for chronic myeloid leukemia (CML). The second-line inhibitors dasatinib and nilotinib provide treatment options for controlling imatinib-resistant CML associated with BCR-ABL kinase domain mutations. However, the T315I mutant of BCR-ABL is resistant to all 3 clinical inhibitors, and is a frequent cause of salvage therapy failure. AP24534 is an oral, multi-targeted kinase inhibitor with activity against native and kinase domain-mutant BCR-ABL, including T315I. We have previously utilized an in vitro mutagenesis-screening assay to successfully predict the profile of mutations that confer resistance to imatinib, dasatinib, and nilotinib in patients. Here we use the in vitro mutagenesis screen to test whether BCR-ABL mutants can emerge in the presence of AP24534. *Methods:* To determine a resistance profile for AP24534, Ba/F3 cells expressing native BCR-ABL were mutagenized with ENU, washed, and plated in the presence of graded concentrations of AP24534 (5-80 nM). For each condition, $4.8x10^7$ mutagenized cells were distributed into 480 wells and observed for growth for 4 weeks. Resistant clones were expanded in the continued presence of AP24534 and sequenced for mutations in the BCR-ABL kinase domain. *Results*: We first established IC_{50} values for inhibition of proliferation of Ba/F3 cells expressing native BCR-ABL (IC_{50}: 0.5 nM) and an extensive panel of imatinib-resistant BCR-ABL mutants (IC_{50} range: 0.5 nM to 35.7 nM) including T315I (IC_{50}: 11.4 nM) and E255V (IC_{50}: 35.7 nM). Parental Ba/F3 cells were not inhibited up to a concentration of 1713 nM AP24534. Corresponding immunoblot analyses confirmed the same rank order for effective inhibition of CrkL phosphorylation in cells expressing native BCR-ABL, the T315I mutant, or the E255V mutant. Inhibition of CrkL phosphorylation was also demonstrated with primary hematopoetic cells from CML patients harboring native BCR-ABL or the T315I mutant. In the mutagenesis screen starting with Ba/F3 cells expressing native BCR-ABL, resistant clones recovered in 10 nM AP24534 expressed native BCR-ABL or one of several imatinib-resistant BCR-ABL mutants (168/1440 wells in 3 independent experiments). By contrast, when the screen was conducted in the presence of 20 nM AP24534, the frequency of outgrowth of escape mutants was extremely low and limited to cells expressing the T315I mutant (2/1440 wells) or the E255V mutant (1/1440 wells). Remarkably, outgrowth was completely suppressed by 40 nM AP24534. *Conclusions*: AP24534 is a potent inhibitor of native BCR-ABL and all tested BCR-ABL mutants, including T315I. Mutagenesis screening reveals that single-agent AP24534 (40 nM) completely suppressed outgrowth of escape mutants. This is in marked contrast to any of the BCR-ABL inhibitors previously profiled in this assay, where outgrowth was evident at the highest tested drug concentrations and complete suppression was observed only when dasatinib or nilotinib was combined with an investigational T315I inhibitor (PNAS 2008; 105: 5507). As sequential BCR-ABL kinase inhibitor therapy has been linked to selection of rare subclones in which 2 mutations occur in the same BCR-ABL molecule, compound mutations are potentially capable of thwarting any of the current clinical BCR-ABL inhibitors, even in combination. Front-line therapy with a pan-BCR-ABL inhibitor could improve the depth and durability of responses by preventing selection of drug-resistant kinase domain point mutants. Our pre-clinical profiling indicates that AP24534 is an important new option in controlling resistance in CML. A phase 1 clinical trial designed to evaluate AP24534 treatment in patients with refractory CML and other hematologic malignancies has recently commenced.

8 Selected posters

8.1. **[1098] Efficacy and Safety of Bosutinib (SKI-606) in Patients with Chronic Phase (CP) Ph+ Chronic Myelogenous Leukemia (CML) with Resistance or Intolerance to Imatinib.** *Cortes at al.* Bosutinib (SKI-606) is an orally bioavailable dual Src/Abl inhibitor demonstrating inhibitory activity against BCR-Abl phosphorylation, and is 200 times more potent than imatinib but with minimal inhibition of platelet-derived growth factor receptor (PDGFR) or c-kit. The phase I portion of this study identified a treatment dose of 500 mg daily and showed evidence of clinical efficacy. The phase II portion of the study to investigate the efficacy and safety of bosutinib in patients (pts) with CP Ph+ CML who have failed imatinib therapy is ongoing. Preliminary data for 283 treated pts, median age 54 yrs (range 18 – 91 yrs) and 52% male are reported. A subset of pts received treatment in addition to imatinib, including interferon

(91 pts), dasatinib (71 pts), nilotinib (7 pts) and stem cell transplant (13 pts). Among pts who failed imatinib (and received no other tyrosine kinase inhibitor treatment), 137 were imatinib-resistant (all received imatinib ≥600mg) and 64 pts were imatinib-intolerant; median duration of bosutinib treatment to date is 7.7 mos (range 0.2 – 28.2 mos) and 4.5 mos (range 0.5 – 21.5 mos), respectively. Among 67 imatinib-resistant pts evaluable for hematological response, 53 (79%) had complete hematological response (CHR). Of 84 imatinib-resistant pts evaluable for cytogenetic response, 34 (40%), achieved a major cytogenetic response (MCyR), including 24 (29%) with a complete cytogenetic response (CCyR). Of 34 pts with MCyR, 31 have maintained their response to date. Of 60 evaluable imatinib-resistant pts, 20 (33%) achieved major molecular response, 10 (17%) of which were complete. Among imatinib-intolerant pts, 22 of 29 evaluable (76%) achieved CHR, and 13 of 22 evaluable (59%) achieved MCyR, including 11 (50%) with CCyR. Of 25 evaluable imatinib-intolerant pts, 7 (28%) achieved major molecular response, 5 (20%) of which were complete. Of 105 pts with baseline samples tested for mutations, 17 different mutations were found in 45 pts (43%). CHR occurred in 5/6 pts (83%) with P-loop mutations and 13/17 (76%) with non-P-loop mutations; MCyR occurred in 3/6 pts (50%) and 11/24 pts (46%), with P-loop and non-P-loop mutations, respectively. Treatment was generally well tolerated. The most common adverse events among treated pts (n=283) were gastrointestinal (nausea, vomiting, diarrhea), these were usually grade 1 – 2, manageable and transient, diminishing in frequency and severity after the first 3 – 4 weeks of treatment. Grade 3 – 4 non-hematologic toxicity occurring in ≥5% of pts were diarrhea (8%), rash (8%) and increased ALT (5%). 27 pts (10%) reported grade 1/2 fluid retention adverse events, including 21 pts with edema, and 6 pts with effusions: 4 pleural, 1 pericardial, and 1 pleural and pericardial. A single patient experienced grade 3 pleural effusion possibly related to bosutinib with concomitant pneumonia and a pre-treatment history of recurrent pleural effusions. Grade 3 – 4 hematologic laboratory abnormalities included thrombocytopenia in 65 pts (23%), neutropenia in 37 pts (13%) and anemia in 17 pts (6%). 124 pts (44%) had at least 1 temporary treatment interruption and 85 pts (30%) had at least 1 dose reduction due to toxicity. 37 pts (13%) have permanently discontinued treatment due to adverse event. Bosutinib is effective in pts with CP CML with resistance or intolerance to imatinib across a range of mutations. Unlike other tyrosine kinase inhibitors, bosutinib does not significantly inhibit PDGFR or c-kit, and this may be responsible for the relatively favorable toxicity profile with few pts experiencing hematologic toxicity or fluid retention.

8.2. **[1102] The Majority of Chronic Myeloid Leukaemia Patients Who Cease Imatinib after Achieving a Sustained Complete Molecular Response (CMR) Remain in CMR, and Any Relapses Occur Early.** *Ross et al.* After 5 years of imatinib treatment 40-50% of chronic myeloid leukaemia (CML) patients will have stable undetectable BCR-ABL by real-time quantitative RT-PCR (RQ-PCR) using strict sensitivity criteria (‘complete molecular response', CMR). Many patients who stop imatinib in CMR will relapse, but small numbers have been reported with sustained CMR after imatinib withdrawal. We designed a non-randomised prospective Phase 2 study of imatinib withdrawal in adult chronic phase CML patients in CMR for ≥2 years (ACTRN012606000118505). Patients were treated in multiple centres around Australia, and RQ-PCR for BCR-ABL was performed centrally: monthly for the first year after imatinib withdrawal, and 2-monthly in the second year. Molecular relapse was defined as a single PCR result above the level of major molecular response (MMR) or any two consecutive positive results. Molecular relapse was treated with imatinib and patients were monitored monthly for 12 months to assess response to re-treatment. Patients were enrolled in two cohorts: imatinib de novo (IM only, n=5) and imatinib after prior interferon therapy (IFN-IM, n=13). The median duration of prior IFN was 39 months. Both cohorts continue to accrue. For all 18 patients the median age at study entry was 58 years; 44% were male. The median duration of imatinib treatment was 60 months (R40-89). The Kaplan-Meier estimate of the rate of sustained CMR after 12 months off treatment was 67% (95% confidence interval 40-85%, see Figure). Ten of 13 IFN-IM patients (77%) remain in CMR, and 7 of these have been in CMR for at least 12 months without treatment (maximum 23 months). The median follow-up in the IM only patients is currently only 7 months (R1-15), and 3/5 remain in CMR. All molecular relapses in both groups have occurred within 5 months of stopping imatinib. The median duration of prior imatinib treatment was not different in the 5 patients with loss of CMR (76 months) versus those in stable CMR (60 months; p=0.59). Among the 5 patients with loss of CMR the median time to molecular relapse was 3 months (range 2-5 months). Two relapsing patients lost MMR, and 3 had detectable BCR-ABL mRNA below this level. No patient has experienced haematological relapse or developed a kinase domain mutation. At last follow-up all 5 relapsing patients had regained CMR after a median of 5 months of re-treatment with imatinib. Patient-specific DNA Q-PCR assays were developed to test whether minimal residual disease (MRD) was detectable in genomic DNA in patients in CMR defined by RQ-PCR for BCR-ABL mRNA. Results are available for 6 patients, 3 of whom have relapsed. One relapsing patient had BCR-ABL DNA detected prior to imatinib withdrawal. In the remaining 2 relapsing patients BCR-ABL DNA was detected

after imatinib withdrawal, but 2-3 months prior to the detection of BCR-ABL mRNA by RQ-PCR. BCR-ABL DNA increased by at least 1-log between the time of the first positive result and the detection of molecular relapse by RQ-PCR. The 3 patients in stable CMR had no detectable BCR-ABL DNA. In conclusion, with close molecular monitoring imatinib withdrawal in stable CMR appears to be safe: currently all patients are either in stable CMR off treatment or back in CMR after re-treatment. Withdrawal of effective treatment outside the setting of a clinical trial is not recommended. Monitoring of MRD by genomic DNA Q-PCR was able to detect molecular relapse prior to mRNA RQ-PCR, and shows promise for the prospective identification of patients at high risk of relapse. There is an apparent dichotomy of response between early molecular relapse and durable CMR, at least in patients treated with imatinib after IFN. It is too early to identify clinical or laboratory factors (such as prior IFN treatment) that may influence the probability of sustained CMR without treatment.

8.3. **[3232] Preliminary Clinical Activity in a Phase I Trial of the BCR-ABL/IGF-1R/Aurora Kinase Inhibitor XL228 in Patients with Ph$^+$ Leukemias with Either Failure to Multiple TKI Therapies or with T315I Mutation.** *Cortes et al.* XL228 is a protein kinase inhibitor with potent activity against wild-type and T315I isoforms of BCR-ABL (wild-type ABL kinase, IC_{50} = 5 nM; ABL T315I, 1.4 nM), Aurora A (3.1 nM), IGF-1R (1.6 nM), SRC (6.1 nM), and LYN (2 nM). A Phase 1 dose escalation clinical trial in patients (pts) with CML or Ph$^+$-ALL who are resistant or intolerant to at least two prior standard therapies (including imatinib, dasatinib, and nilotinib) or have a known BCR-ABL T315I mutation is ongoing. XL228 is administered as a 1-hour IV infusion either once weekly or twice weekly. Twenty-seven pts have been enrolled into six cohorts with the once-weekly dosing schedule (dose range from 0.45 mg/kg to 10.8 mg/kg). All pts have failed prior imatinib therapy, and received nilotinib, dasatinib, and other therapies The majority of pts harbor mutations in BCR-ABL, with the most common mutations being T315I (n=10), F317L (n=7), and V299L (n=3). The maximum administered dose (MAD) of once-weekly IV dosing of XL228 is 10.8 mg/kg. Dose escalation of pts in the twice-weekly dosing schedule at an initial XL228 dose of 3.6 mg/kg on Days 1 and 4 of each week is ongoing. XL228 has been generally well-tolerated. Dose limiting toxicities observed with once-weekly dosing included Grade 3 syncope and hyperglycemia in two pts dosed at 10.8 mg/kg. Grade 2 adverse events reported to be possibly related to XL228 in the once-weekly dosing schedule were usually transient and manageable, and included hyperglycemia, fatigue, nausea, vomiting, and bradycardia. Pharmacokinetic analysis across five cohorts treated with once-weekly dosing of XL228 demonstrated an approximately dose-proportional exposure, with a mean terminal half life of 15 to 38 hours. In the 7.2 mg/kg cohort, the C_{max} of approximately 13 μM exceeds the IC_{50} for modulation of phospho-CrkL levels determined in mouse K562 xenograft pharmacodynamic studies. Peak exposures of XL228 in the 7.2- and 10.8-mg/kg cohorts were associated with inhibition of peripheral blood leukocyte CrkL phosphorylation in several patients, including three harboring the T315I mutation. Transient increases in mean plasma glucose levels (up to three fold) and mean insulin levels (up to 40 fold) post-infusion are coupled, dose-related, and imply inhibition of the IGF1R and IR pathways by XL228. Preliminary evidence of clinical activity has been observed in pts treated at doses of 3.6 mg/kg and higher, including stable or decreasing white blood cell count and/or platelet count within 2 months (14 pts, 5 with T315I), and/or >1 log reduction in BCR-ABL levels by QPCR within 3 months (3 pts, 2 with T315I). Pts in the 7.2 mg/kg and higher cohorts have been followed a minimum of 1 month to a maximum of 4 months at the time of abstract submission. XL228 shows potential for treating drug-resistant CML and Ph$^+$-ALL, including pts harboring the T315I gatekeeper mutation.