ORAL ABSTRACTS SESSION: 12:00 PM-1:30 PM

631. Chronic Myeloid Leukemia: Biology and Pathophysiology, excluding Therapy: Basic and Translational Studies to Improve Treatment Outcomes and Identify Immune Mediators of Treatment-free Remission

Saturday, December 7, 2019: 12:00 PM-1:30 PM
Tangerine 2 (WF2), Level 2 (Orange County Convention Center)

Moderators:
Daniela S. Krause, MD, University of Frankfurt and Rob Welner, PhD, UAB

187 CD93-Signaling Regulates Self-Renewal and Proliferation of Chronic Myeloid Leukemia Stem Cells in Mice and Humans and Might be a Promising Target for Treatment

Carsten Riether, PhD

Background: The introduction of BCR/ABL-specific tyrosine kinase inhibitors (TKIs) more than two decades ago revolutionized chronic myelogenous leukemia (CML) therapy. The majority of CML patients treated with TKIs obtain durable cytogenetic and molecular responses. However, only a subgroup of these patients can successfully discontinue TKI therapy and maintain a treatment-free remission (Laneuville et al. 2011). TKI-resistant leukemia stem cells (LSCs) persist in the majority of patients at low levels over a prolonged period. These quiescent, self-renewing LSCs in the BM are the major cause of relapse after drug discontinuation (Holyoake et al, 2017). The selective elimination of LSCs requires the definition of unique signaling pathways that promote self-renewal of LSCs but not of normal HSCs. Based on the documented expression of CD93 on LSCs (Kinstrie et al, 2015), the aim of the present study was to investigate the role of the cell surface receptor CD93 in the regulation of self-renewal of human and murine CML LSCs and its contribution to disease development and progression.

Methods and Results: We found CD93 expression on LSCs and leukemia progenitor cells but not on more differentiated leukemia granulocytes in a murine retroviral lineage-negative Sca-1+ c-kit+ (LSK) transduction/transplantation CML model. Next-generation sequencing analysis revealed that Cd93-/- LSCs have a silenced gene expression signature particularly in genes involved in the regulation of gene expression, stem cell maintenance and proliferation. Out of the 1120 genes differentially expressed between BL/6 and Cd93-/- LSCs, 1108 genes were down-

regulated. In contrast, naïve BL/6 and Cd93-/- hematopoietic stem cells (HSCs) did not display a dysregulation in these pathways. Functionally, CD93-deficiency in LSCs resulted in impaired self-renewal, reduced LSC frequencies in vitro (at least by a factor of 100, $P<0.001$) and in the incompetence to induce and propagate CML in mice.

To study whether CD93-signaling in LSCs relies on ligand-binding to the extracellular domain of CD93, we generated an extracellular domain deletion mutant of CD93 (mCd93intra). Comparable to transduction with full-length mCd93, the expression of Cd93intra restored colony formation of Cd93-/- LSCs in vitro, suggesting that the maintenance of LSC self-renewal is independent of ligand-binding to the extracellular domain of CD93. Furthermore, analysis of the sub-cellular localization of CD93 in CML cells using a lentiviral expression vector encoding for AcGFP1-N1-Cd93 demonstrated nuclear localization of the CD93 intracellular domain (ICD). SCY1 like pseudokinase 1 (SCYL1), a regulator of gene transcription, directly interacts with the highly charged juxta membrane domain of the cytoplasmic tail of CD93 (Bohlson et al, 2005). Silencing of Scyl1 significantly reduced colony formation of BL/6 but not Cd93-/- LSCs in vitro suggesting that the ICD of CD93 regulates gene transcription via Scyl1 in CML LSCs.

To discover compounds that affect LSC function similarly as genetic CD93 blockade, we performed a compound screen using the FDA approved drug library V2. The antiemetic agent metoclopramide, which is widely used in clinical routine to reduce nausea in cancer patients, was one very promising candidate identified in the screen. Metoclopramide treatment reduced clonogenic potential of CD93-competent LSCs to comparable levels as CD93-deficient LSCs in vitro without further affecting colony formation of CD93-deficient LSCs.

Analysis of LSCs from newly diagnosed CML patients similarly demonstrated that CD93-signaling induces the expression of genes associated with proliferation and stemness, resulting in an increased clonogenic potential in vitro. In addition, colony formation and re-plating capacity in semisolid cultures of human CD34+CD38- LSCs was significantly impaired by metoclopramide at a pharmacological concentration of 0.1mM compared to control treatment.

Conclusions: Overall, these results indicate that CD93-siganling is an important regulator of stemness and proliferation of human and murine CML LSCs. Furthermore, this study identifies expression of CD93 by LSCs as promising novel target for the treatment of CML.

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188 Combining the Allosteric ABL1 Inhibitor Asciminib (ABL001) with Ponatinib Suppresses Emergence of and Restores Efficacy Against Highly Resistant BCR-ABL1 Compound MutantsClinically Relevant Abstract

Christopher A. Eide

BCR-ABL1 point mutation-mediated resistance to tyrosine kinase inhibitor (TKI) therapy in Philadelphia chromosome-positive (Ph+) leukemia is effectively managed with several approved drugs, including ponatinib for BCR-ABL1T315I-mutant disease. However, for those patients who acquire BCR-ABL1 compound mutations (multiple mutations in the same BCR-ABL1 molecule), therapy options are extremely limited. Asciminib (formerly ABL001) is a recently developed allosteric inhibitor targeting the myristoyl-binding pocket of ABL1 kinase with activity against many imatinib-resistant BCR-ABL1 mutants, including T315I. We profiled asciminib against a panel of BCR-ABL1 single and compound mutants expressed in murine Ba/F3 cells. Asciminib potently inhibited the proliferation of most imatinib-resistant BCR-ABL1 point mutations tested, with the notable exception of substitutions at position F359, which conferred high levels of resistance. Consistent with this finding, next-generation sequencing of BCR-ABL1 in five patients with evidence of clinical resistance to asciminib revealed three patients with expansion of variants of position F359 on treatment. Cell-based mutagenesis screens starting from Ba/F3 cells expressing native BCR-ABL1 revealed a resistance profile for asciminib largely centered around residues of the myristoyl pocket, with these mutants remaining sensitive to approved ATP-site ABL1 TKIs. Combining asciminib with ATP-site TKIs enhanced target inhibition and suppression of resistant BCR-ABL1 point mutant outgrowth in Ph+ clinical isolates and cell lines. However, despite its unique binding mode, asciminib was ineffective against all tested BCR-ABL1 compound mutants. In contrast, combining asciminib with ponatinib re-sensitized even the problematic, currently untreatable T315I-inclusive compound mutants at clinically achievable concentrations, which was not achieved combining asciminib with other approved ATP-site TKIs. Additionally, the combination of asciminib with ponatinib resulted in suppression of T315I-inclusive compound mutant resistant clones using in vitro mutagenesis screens and significantly prolonged survival compared to either single agent in an in vivo T315I-inclusive compound mutant mouse xenograft model. Molecular dynamics-based structural modeling were performed and offer further insight into the mechanism of this combination's efficacy. Taken together, our findings support combining asciminib with ponatinib as a treatment strategy for improved management and mitigating the emergence of highly resistant BCR-ABL1 compound mutations in patients with Ph+ leukemia.

189 The AHI-1-BCR-ABL-DNM2 Complex Mediates Mitochondrial Dynamics in Drug-Resistant BCR-ABL+ Cells

Ryan Yen, BSc

Chronic myeloid leukemia (CML) is driven by the BCR-ABL1 oncoprotein with constitutively active protein-tyrosine kinase activity, perturbing multiple signaling pathways. Although therapies with tyrosine kinase inhibitors (TKIs) can effectively treat early phase CML, relapses and emergence of TKI resistance are problematic, due to BCR-ABL kinase domain mutations and TKI unresponsive quiescent leukemic stem cells (LSCs). These observations point towards a need for alternate treatment strategies to prevent the development of resistant LSCs. We previously demonstrated that Abelson helper integration site-1 (AHI-1) is a highly deregulated protein in CML LSCs and that its WD40-repeat domain physically interacts with BCR-ABL, enhancing leukemia-initiating activity. AHI-1 also contains an SH3 domain, which mediates TKI resistance in LSCs. This domain interacts with dynamin-2 (DNM2) and forms a complex with BCR-ABL, to enhance the phosphorylation and activity of DNM2. The AHI-1-BCR-ABL-DNM2 complex is shown to regulate leukemic properties in patient LSCs, including increased ROS production, endocytosis and autophagy. Interestingly, deletion of the Ahi-1 SH3 domain (Ahi-1 SH3Δ) results in a defect in Ahi-1 localization, with most being present in the nucleus. To test whether Ahi-1 SH3 domain activity directly affects cytoplasmic anchoring and localization, we have generated two Ahi-1 mutants, using site-directed mutagenesis: a mutation in the key tryptophan residue (W939A) involved in SH3 domain binding and in a non-conserved surface residue (M906A), as a negative control, based on the crystal structure of the AHI-1 SH3 domain. Interestingly, the cytoplasm-to-nucleus signal ratio of Ahi-1 W939A was significantly reduced compared to the negative control or wildtype Ahi-1, as assessed by immunofluorescence and confocal microscopy (70% reduction, $p<0.0001$), indicating that changes in localization of Ahi-1 SH3Δ may result in disruption of the complex and allow for new interactions with nuclear proteins. Investigating changes in the proteome may help uncover downstream effects of the AHI-1-BCR-ABL-DNM2 complex and its biological role in mediating TKI resistance. Advanced antibody microarray analysis was then used to investigate differences in the proteome and phosphorylation landscape of BCR-ABL+ cells co-transduced with wildtype Ahi-1 or Ahi-1 SH3Δ. This system quantifies the differences in expression and phosphorylation states of key signaling proteins simultaneously, using 878 antibodies in duplicate. Twenty leads were identified by the following criteria: a large signal difference of at least 1.5-fold change, high signal strength for high expression, and low error between duplicates. These leads were validated by Western blot analysis and several of them were confirmed. Particularly, phosphorylation of cyclin-dependent kinase 1 (CDK1), a key player in cell cycle control and mitochondrial dynamics, was greatly reduced in cells expressing wildtype Ahi-1 compared to Ahi-1 SH3Δ, indicating that AHI-1-mediated phosphorylation changes in CDK1 may contribute to regulation of mitochondrial functions. Indeed, BCR-ABL-transduced cells co-expressing wildtype

Ahi-1 showed increased mitochondria potential in response to TKI treatment or serum starvation, in MitoTracker analysis ($p<0.05$). However, this was not observed in BCR-ABL-transduced cells co-expressing the Ahi-1 SH3Δ mutant. A similar trend was also observed in immunofluorescence confocal microscopy analysis of the mitochondrial importer receptor, TOM20. To further study the role of DNM2 in mediating mitochondrial dynamics associated with AHI-1 and BCR-ABL, CRISPR-Cas9 mediated DNM2 knockdown was performed in TKI-resistant cells, using two different DNM2-targeting guide RNAs; these resulted in significant reduction in DNM2 (78% & 75%) in Western blot analysis. The knockdown cells showed a reduction in viability (60% reduction) and increased sensitivity to TKI treatment compared to the control (90% vs. 30% reduction) after 48 hours and changes in mitochondrial activity were also observed in these cells. These results support a role for mitochondrial dynamics in the AHI-1-BCR-ABL-DNM2 complex-mediated TKI response and that targeting key biological processes regulated by the AHI-1-BCR-ABL-DNM2 complex and its pathways may lead to new therapeutic strategies to overcome TKI resistance in CML.

190 BCR-ABL1 p190 in CML: A Minor Breakpoint with a Major Impact

Shady Adnan Awad, MD

Introduction: The oncoprotein Bcr-Abl has two major isoforms, depending on the breakpoint in BCR gene, p190 and p210. While p210 is the hallmark of chronic myeloid leukemia (CML), p190 occurs in the majority of Philadelphia-positive acute lymphoblastic leukemia (Ph+ ALL) patients. p190 occurs as a sole transcript in 1-2% CML patients, associated with distinct features like monocytosis and frequent additional cytogenetic abnormalities (ACA) at diagnosis. It also confers a risk of treatment failure and progression in chronic phase (CP) CML patients. However, the underlying mechanisms are largely unknown. Here we explore the characteristics of p190 and p210 in CML and ALL patients using next generation sequencing, phospho-flowcytometry and high throughput drug testing.

Patients and methods: Peripheral blood mononuclear cells (PMNC) were collected at diagnosis from four CP-CML patients harboring p190 isoform from Helsinki University Hospital. Genetic alterations were identified by whole exome sequencing. RNA sequencing was employed to analyze transcriptional profiles of p190 CML (n=3) in contrast to p210 CML patients (n=4). A thorough transcriptional, phosphorylation and drug sensitivity profiling were applied to five p190- and three p210-expressing Ph+ALL patients. Expression alterations were further characterized in two cell line models mimicking BCR-ABL positive leukemia (Ba/F3 and HPCLSK). Phosphorylation profiles were analyzed by flowcytometry and phospho-array (Tyrosine Phosphorylation ProArray, Full Moon Biosystems). For drug sensitivity and resistance testing (DSRT), a custom plate set comprising 75 approved and investigational oncology drugs was used for patient samples and more extensive 528-drugs plates were used for the cell lines.

Results: CML patients with p190 had a median age of 72.5 years at the diagnosis (range: 50-80) and all received imatinib as a frontline treatment. Only one patient achieved a fluctuating major molecular response (MMR) by 12 months while the rest of the patients showed primary resistance to treatment and were shifted to a 2nd line TKI, nilotinib (n=2) or proceeded to HSCT (n=1). By exome sequencing we identified 26 variants in p190-CML samples (median per patient=7, range: 2-10), including variants in ASXL1, DNMT3A and KDM4D genes. RNA-sequencing analysis identified 19 and 97 dysregulated genes (Q <0.05) between p190- and p210 in CML and Ph+ ALL cells respectively. In CML, enrichment analysis revealed upregulation of TNF, interferon (IFN), IL1-R and Toll-like receptor (TLR) signaling, TP53-related, cell cycle and apoptosis pathways. Among Ph+ ALL samples, many CML-related genes were upregulated in samples encompassing p210 while IFN-, TP53- and cell cycle-related molecules were upregulated in p190 samples. p190 samples exhibited hyper-phosphorylation of Src kinase compared to p210 samples. DSRT results also revealed increased sensitivity of primary Ph+ ALL-p190 cells to Src-inhibitors (dasatinib and saracatinib), glucocorticoids and MDM2 inhibitors/TP53 activators (SAR405838 and idasanutlin). Regarding cell lines, Ba/F3-p190 showed the upregulation of interferon signaling pathways compared to p210. Src was also hyperphosphorylated in both Ba/F3 and HPCLSK p190 models. In addition to glucocorticoids and Src-inhibitors, compounds blocking the activity of the inhibitors of apoptosis protein (IAP) family were highly effective at reducing the viability of p190 compared to p210 cells in both cell lines.

Conclusions: In CML, p190 isoform of BCR-ABL1 is associated with distinct features and should be considered as a high-risk group. Combining clinical, genomic, phosphorylation and drug sensitivity data, we demonstrated that p190 activates specific cancer pathways, notably Src signaling and interferon pathways. Data also suggests that CML patients with p190 could benefit from broad spectrum TKI with Src inhibiting activity or combination of TKI with MDM2- or IAP-inhibitors.

191 Identification of Immunological Parameters Related to Relapse in Patients with Chronic Myeloid Leukemia on Treatment-Free Remission

Valentín Garcia-Gutiérrez, MD, PhD

Background: After long-term treatment with tyrosine kinase inhibitors (TKIs), an important proportion of patients with chronic myeloid leukemia (CML) achieve and maintain a deep molecular response (DMR) that allows them to stop treatment indefinitely. However, approximately 50% of these patients on treatment-free remission (TFR) experience relapse by undetermined reasons. It has been described that TKIs may induce a potent antileukemic response that conditions the outcome of the discontinuation. Our objective was to analyze different immune parameters that could be used as biomarkers of safer treatment discontinuation.

Objectives: To determine the modulation of immune biomarkers that could be related to current treatment with TKIs on patients with CML ("On TKI") or to successful TFR in patients that maintain DMR after treatment withdrawal ("Off TKI") or to relapse after TFR on patients that lost DMR.

Materials & methods: We analyzed by flow cytometry the peripheral blood mononuclear cells (PBMCs) from 45 patients with CML "On TKI" for at least 9 months (imatinib (11), nilotinib (9), dasatinib (20) or bosutinib (5)), 17 patients "Off TKI" for at least 7 months who kept DMR at the moment of sampling (last TKI before TFR: imatinib (7), nilotinib (6), dasatinib(4)), 7 patients "Off TKI" for at least 1 year and 4 months who relapsed during TFR (samples from 3 patients were taken previous to TKI reintroduction (on relapse); samples from 4 patients were taken after they restarted treatment with TKIs ("On TKI" relapse), 4 patients with recent diagnosis of CML still untreated, and 20 healthy donors as basal control.

Results:

1) Patients characteristics are shown in table 1.

2) Treatment with TKIs induced an increase of 8.6±1.2% ((p<0.001) in CD56+ Natural Killer (NK) cells regarding healthy controls. This increase was sustained in patients "Off TKI" during successful TFR but it was reduced 9.5±1.4% in patients "Off TKI" on relapsed CML. This cell population was not recovered in patients on relapse even after restarting TKI treatment. 3) A population of NK cells with cytotoxic phenotype CD3-CD56+CD16+ was increased 8.1±2.8% in "Off TKI" patients during TFM, regarding patients on treatment. This population was reduced 12±3.8% in patients "Off TKI" who relapsed CML but increased 18.8±6.7% once treatment was restarted. 4) The expression of NKG2D - a major recognition receptor for the detection and elimination of cancerous cells – in NK cells was reduced 29.7±1.5% in patients "Off TKI" who relapsed during TFR, regarding "Off TKI" patients who kept DMR. This cell population was not recovered in patients on relapse even after restarting TKI treatment. 5) Populations of cytotoxic cells CD8+TCRgd+ and CD8-TCRgd+ were respectively increased 19±3.9% (p<0.0001) and 6.1±1.0% (p<0.05) in "Off TKI" patients regarding healthy controls but they were reduced 17.95±1.3% and 7.8±0.1%, respectively, in "Off TKI" patients who relapsed CML. 6) Expression of CD86, which has been related to poor prognosis, was increased 9.2±1.0% in patients "Off TKI" with relapsed CML, regarding to patients who maintained DMR on TFR.

Conclusions: Characterization of the immune response developed in patients with CML due to treatment with different TKIs may be relevant to predict the success of treatment discontinuation. We have determined that the modulation of the expression of the following markers may be used as potential predictors of TFR outcome: CD56+<4%; CD3-CD56+CD16+>7%; CD56+NKG2D+>24%; CD8+TCRgd+>7%; CD8-TCRgd+>4%; CD86+<20%. These biomarkers need to be validated in a larger cohort of patients.

Table 1. Patient characteristics.

		On TKI	OFF TKI maintaining DMR	OFF TKI losing DMR	Newly CML patients
Patients, n		45	17	7	4
Male/female		17/28	11/6	5/2	2/2
Sokal (L/I/H)*		39/1/5	16/1/0	7/0/0	4/0/0
Response at sampling	**CCyR**	42	17	5	NA**
	MMR	35	17	5	NA
	DMR	25	17	0	NA

L/I/H: Low/Intermediate/High. **NA: not applied.*

Table 2. Expression of immunological parameters showed by different groups of patients with CML.

	% expression					
	CD56+	**CD3-CD56+CD16**	**CD56+ NKG2D+**	**CD8+ TCRγδ+**	**CD8- TCRγδ+**	**CD86+**
HEALTHY DONORS	10,4±0,6	11±1,1	49,7±3,5	5,5±0,7	5,7±0,9	5,3±0,5
ON TKI	19±1,4	12,9±1,7	27,8±2,9	11,8±1,2	6,5±0,9	13,4±1,2
OFF TKI	13,8±1,7	19,1±3,9	53,6±6,1	24,5±4,6	11,8±1,9	10,9±1,2
Relapse	4,3±0,3	7,1±0,1	23,9±4,6	6,5±3,3	4±2,0	20,1±0,2
ON TKI after relapse	2,9±1,1	25,9±6,8	17,2±4,7	9,7±5,0	4,3±2,1	1,7±0,2
No treatment	6±0,4	2,4±0,5	20,1±1,9	4,1±1,3	3,3±1,0	14,2±6,5

192 Optimum Imatinib Exposure Have Possibility of Leading to Appropriate Immune Response after Imatinib Discontinuation in CML Patients

Yuki Fujioka, MD, PhD

Introduction: Imatinib, the first tyrosine kinase inhibitor (TKI), has dramatically improved the prognosis of chronic myeloid leukemia (CML) patients. Recently, many trials of TKI discontinuation revealed that approximately 40% to 60% of CML patients who treated long time TKI therapy reached the treatment free remission (TFR), thus now TFR is proposed as one of the goals in CML treatment. Achieving deep molecular response (DMR) by TKI therapy is a minimum requirement of challenge to TKI discontinuation in CML patient, actually CML patients with molecular residual disease (MRD) showed worse consequence than undetectable MRD (IJH 2017). On the other hand, it was known that some patients have continued TFR with detectable BCR-ABL fusion gene, these patients hadn't shown indubitable molecular relapse while BCR-ABL+ malignant cells continued to

exist for prolonged time. We hypothesized that the malignant cells were eliminated by host immune systems in these fluctuated patients. Here, we focused on T-cell response, so we analyzed T-cell related markers to identify biomarkers that can predict patients which can continue TFR or not in Japanese CML patients. Furthermore, we confirmed the action of imatinib for T-cell response in vitro.

Methods: Japanese CML patients treated with imatinib for at least three years and confirmed in DMR for at least two years were eligible. Patients who received other TKI or stem cell transplantations were excluded. Patients were re-confirmed in MR4.5 before discontinue imatinib and they were sampled peripheral blood at pre- and 1, 3 months after stopping imatinib (figure 1). Peripheral blood mononuclear cells (PBMCs) were subjected to staining with T-cell markers and analyzed by mass cytometry and flowcytometry. Plasma were subjected to detecting Imatinib trough concentrations. Purchased PBMCs of healthy individuals were cultured and analyzed by flowcytometry in vitro assay.

Results: Samples of 68 CML patients were analyzed. We classified these CML patients into two groups (Non-retreatment and Retreatment groups) by clinical courses after stopping imatinib (figure 2). Frequency of CD4+ T cells and CD8+ T cells in CD3+ T cells were no difference between both groups. FoxP3+CD4+ regulatory T cells (Treg) were also no difference between both groups, but kinetics of Treg, especially Fraction II (Fr.II : FoxP3hiCD45RA-) of Treg from Pre-stopping imatinib to 1 month after stopping imatinib significantly increased in non-retreatment groups. Kinetics of Treg / CD8+ T cells ratio also significantly increased in non-retreatment groups, and predicted curve made by these kinetics of each groups were significant (figure 3). The expression of PD-1 or other suppressive co-stimulatory molecules in CD8+ T cells of non-retreatment groups at after stopping imatinib had tendency to decrease. Phosphorylated LCK in CD8+ T cells of non-retreatment groups at after stopping imatinib had tendency to increase. Next, we did in vitro assay to confirm the effect of pre-treatment of imatinib in imatinib free T cells. Pre-treatment of imatinib suppressed the proliferations of Treg Fr.II after TCR stimulation dose dependently, but not CD8+ T cells (figure 4). Frequency of phosphorylated LCK in Treg Fr.II increased after TCR stimulation even if pre-treated imatinib at reasonable dose, but didn't increased under the condition of high dose imatinib.

Conclusion: Treg population and Treg / CD8+ T cells ratio in PBMCs elevated after stopping imatinib in non-retreatment groups of CML patients. Population of CD8+ T cells showed no differences in two groups but CD8+ T cells were tending to activate after stopping imatinib in non-retreatment groups. These data indicate that the kinetics of Treg after stopping imatinib connect with the immune response of imatinib discontinued CML patients. In vitro data indicate that Treg were more sensitive for imatinib treatment than CD8+ T cells, so kinetics of Treg may possibly become the biomarker of ability of immune responses. Our data suggested that optimum imatinib exposure induce appropriate immune responses leading good prognosis, and excess imatinib exposure induce exhaust immune responses leading poor prognosis.

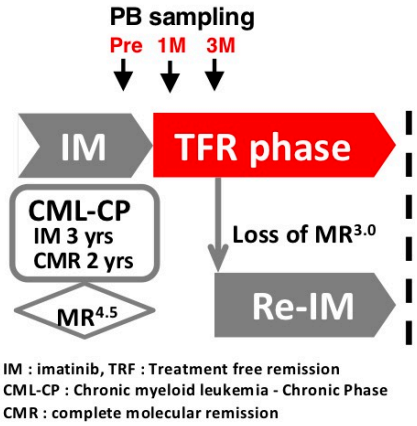

Fig.1 Schema of our trial.

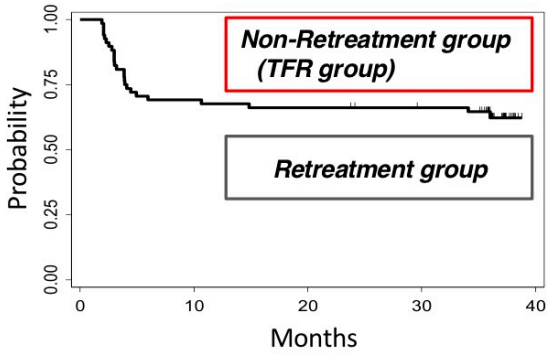

Fig.2 Kaplan-Meier curve of TFR.

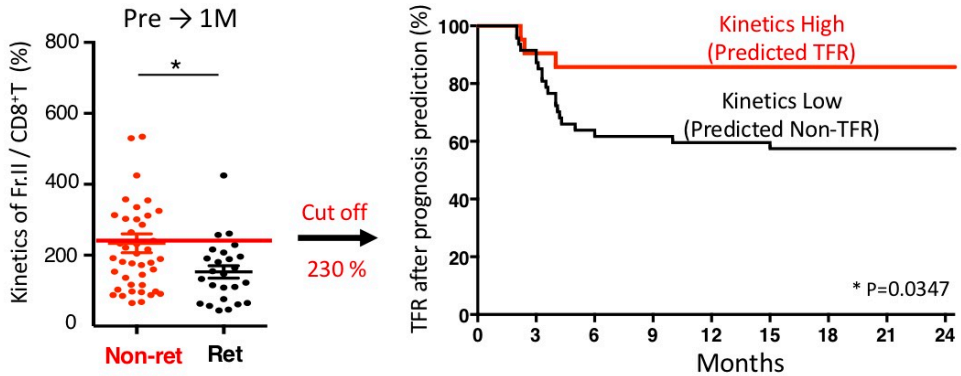

Fig.3 Kinetics of Treg / CD8+T cells makes prognosis prediction curve.

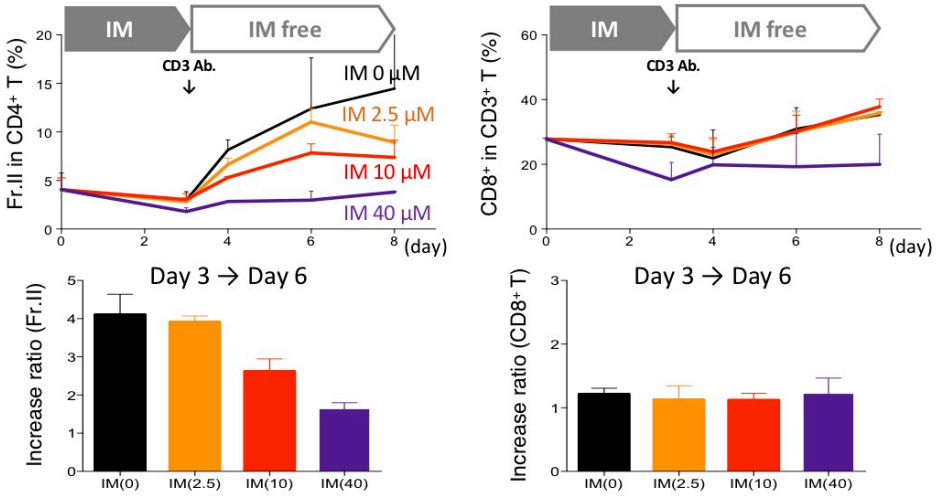

Fig.4 Kinetics of Treg cells and CD8+T cells after TCR stimulation with imatinib pre-treat.