administered a blood test for SARS-CoV-2 spike(S) protein-specific antibodies by a semi-quantitative immunoassay (ELISA) which was considered positive at cut-off index (CI)>1. Other available tests for antibody detection were allowed as well. **Results:** Nighty-five chronic phase CML patients with a median (Me) age 54 years (range 29-89), received Sputnik V (DEC.2020–JUL.2021), 40(42%) were males. Me CML duration was 8 years (range 0-20), 75(79%) patients received TKIs at vaccination, 20(21%) were off-therapy: 17(18%) in treatment-free remission, 3(3%) with CML onset. Seventeen (18%) patients had a prior history of COVID-19. AEs were reported in 53(56%) patients: local pain/discomfort -30(31.5%), weakness/drowsiness -29 (30.5%), fever and/or chills -28(29%), other AEs -10(12%): headache, heartbeat, limbs/back pain, herpes reactivation. General reactions stopped in 1-2 days. No severe or life-threatening AEs were observed. Antibodies were detected in 66(93%) of 71 patients by any method, Me time after 2nd injection was 31 days (range 5-179). ELISA test was positive in 48(94%) of 55 tested patients with Me CI 7.7 (range 1.1-12), consistent with values of healthy people. Three of 7 negative by ELISA patients (Me age 58 years (range 40-70)) revealed antibodies by other tests with levels slightly above threshold. A very weak reverse correlation of the antibody levels with post-vaccination time (r =- 0.32) and with age (r =- 0.28) was observed. **Conclusions:** Sputnik V vaccine showed no unexpected or severe AEs in CML patients. Seroconversion rate of about 93-94% was close to the 3-phase trial data (94-97%). No strong age-dependent/time-dependent correlation of antibody levels was found in the tested time period. Sputnik V vaccination is safe and acceptable in CML patients. **Keywords:** CML, chronic myeloid leukemia, COVID-19, vaccination, vaccine, SARS-CoV-2

CML-155

Prognostic Markers of the Effectiveness of Tyrosine Kinase Inhibitors in Third-Line Therapy of Chronic Phase Chronic Myeloid Leukemia Patients: Data From a Multicenter Study

Tamara Chitanava MD[1], Vasily Shuvaev PhD[2], Iuliia Matvienko MD[1], Irina Martynkevich PhD[2], Sergey Voloshin PhD[2], Elizaveta Efremova MD[2], Ekaterina Mileeva MD[2], Mikhail Fominykh PhD[3], Anna Kersilova MD[4], Elena Koryagina MD[5], Natalia Ilyina MD[5], Natalia Dorofeeva MD[6], Nadezhda Medvedeva MD[6], Anna Klimovich MD[6], Tatiana Shneider MD[7], Svetlana Stepanova MD[7], Natalia Polezhajkovskaya MD[7], Nadiya Siordiya MD[1], Eugenia Sbityakova MD[1], Natalia Lazorko MD[1], Elena Tochenaya MD[1], Dmitry Motorin PhD[1], Nadezhda Shnalieva MD[1], Yulia Alekseeva PhD[1], Darina Zammoeva MD[1], Elza Lomaia PhD[1]

[1]Personalized Medicine Centre, Almazov National Medical Research Centre, Saint-Petersburg, Russian Federation. [2]Russian Research Institute of Hematology and Transfusiology, Saint-Petersburg, Russian Federation. [3]Limited Liability Company The Scandinavia "AVA-PETER Clinic" The Central Department of the Scandinavia Clinic (The Liteyniy Clinic), Saint-Petersburg, Russian Federation. [4]City Clinical Hospital n.a. V.V. Veresaev of the Moscow Department of Healthcare, Moscow, Russian Federation. [5]City Clinical Hospital No.15, Saint-Petersburg, Russian Federation. [6]City Clinical Hospital No.31, Saint-Petersburg, Russian Federation. [7]Leningrad Regional Clinical Hospital, Saint-Petersburg, Russian Federation

Context: Despite success in chronic myeloid leukemia (CML) treatment there are unmet needs third-line therapy. **Objective:** To determine the efficacy and factors influencing the long-term outcomes of third-line therapy with tyrosine kinase inhibitors (TKIs). **Design:** Multicenter retrospective study was conducted in 2019. **Patients or Other Participants:** All adult 73 pts, including 26(35%), CML chronic phase (CP) pts treated with TKIs in third line (TKI-3L) without complete cytogenetic response (CCyR) at baseline were included in the study. The me duration of CML from diagnosis to baseline was 63(10–314) mos. **Main Outcome Measures:** The rate of CCyR (equal to *BCR::ABL* transcript level <1%) and overall survival (OS) were assessed. Univariate and multivariate logistic regression analyses assessed risk factors for CCyR achievement. For a scoring system, selection of predictors was performed using L2-regularization after standardization of quantitative variables. **Results:** The me duration of TKI-3L therapy was 14(1–120) mos, the me time of follow-up from initiation of TKI-3L was 25(3–136) mos. CCyR was achieved in 22/73(30%) pts. Both in univariate and multivariate regression analysis factors influenced on CCyR achievement were absence of CCyR at baseline (OR 0.26[95% CI, 0.08–0.85]; p=0.0252), no CCyR on TKI-1L and TKI-2L (OR 0.21[95% CI, 0.04–0.99]; p=0.0502), as well as pt's age at baseline per every 10 years (OR 1.52[95% CI, 0.61–3.8]; p=0.369). Score system was set up based on these factors with scores 7, 8, 1 score per 10 years respectively. ROC analysis divided pts into three groups: low risk (score ≤9, n=22), intermediate-risk (score 10-15, n=27) and high risk (score ≥16, n=24). The low-risk group had a significantly higher rate of CCyR on TKI-3L compared to intermediate or high-risk groups (14/22[64%] vs 7/27[26%] vs 1/24[4%], p<0.05). There were 19(26%) deaths. Estimated 1-year and 5-year OS was 95% and 65% respectively. All CML-related deaths(n=14), as well as transformation to BC (n=13), occurred in intermediate- and high-risk groups. **Conclusions:** Nearly a third of pts obtained CCyR on TKI-3L in our study. Most pts were alive at 5 years. Younger pts with any CyR on previous TKIs and at baseline had favorable prognosis. The use of TKI-3L is justified in low-risk groups and may be used in transplant-eligible pts. **Keywords:** chronic myeloid leukemia, chronic phase, complete cytogenetic response, tyrosine kinase inhibitors, third-line therapy

CML-164

Dihydroorotate Dehydrogenase Inhibition Reveals Metabolic Vulnerability in Chronic Myeloid Leukemia

Mohammad Houshmand PhD[1], Nicoletta Vitale PhD[1], Francesca Orso PhD[1], Alessandro Cignetti MD[2],

Ivan Molineris PhD[1], Valentina Gaidano MD, PhD[2], Stefano Sainas PhD[1], Marta Giorgis PhD[1], Donatella Boschi PhD[1], Carmen Fava MD[1], Alessandra Iurlo MD[3], Elisabetta Abruzzese MD[4], Massimo Breccia MD[5], Olga Mulas MD[6], Giovanni Caocci MD[6], Fausto Castagnetti MD[7], Daniela Taverna PhD[1], Fabrizio Pane MD[8], Marco Lolli PhD[1], Paola Circosta PhD[1], Giuseppe Saglio MD[1]

[1]University of Turin, Turin, Italy. [2]Mauriziano Hospital, Turin, Italy. [3]Foundation IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy. [4]Eugenio Hospital, Rome, Italy. [5]University of Sapienza, Rome, Italy. [6]University of Cagliari, Cagliari, Italy. [7]University of Bologna, Bologna, Italy. [8]University of Naples "Federico II", Naples, Italy

Context: There are chronic myeloid leukemia (CML) patients who show resistance to TKI therapy and are prone to progress to more advanced phases of the disease, and new therapeutic approaches are needed. Our study shows that CML cells are vulnerable to dihydroorotate dehydrogenase (DHODH) inhibition mediated by Meds433, a potent DHODH inhibitor developed by our group. **Objective:** Pyrimidine supply in resting and differentiated cells primarily relies on the salvage pyrimidine pathway, which is energetically affordable. This level of nucleotide production for fast-proliferating leukemic cells is insufficient, and they have to fulfill their needs via the de novo pathway. In this study, we shed light on the role of DHODH inhibition in CML and how it can be a promising approach for targeting leukemic cells. **Design:** Sixty bone marrow samples and peripheral blood of newly diagnosed CML patients and 4 TKI-resistant patients were collected. *In-vivo*, *in-vitro*, and *ex-vivo* experiments were performed on primary CML $CD34^+$ and various CML cell lines. **Results:** Our data showed that DHODH is highly active in CML stem/progenitor cells, which supports a high proliferation capacity. Meds433, by targeting DHODH enzyme activity, induced apoptosis, cell growth, and cell cycle arrest in leukemic cells. Meanwhile, the administration of Meds433 reduced tumor growth and tumor burden in treated mice. Interestingly, the addition of exogenous uridine rescued all of the biological effects caused by DHODH inhibition, demonstrating the selectivity of Meds433. Based on RNA-seq data, most upregulated gene sets were related to apoptosis and immune response and most downregulated gene sets were related to MYC targets and metabolism pathways, which was confirmed by metabolic profile analysis. Also, we found that glutamic pyruvic acid transaminase 1 (GPT1) is among the top downregulated genes after treating CML cells with Meds433, and overexpressing GPT1 in CML cells interfered with the effect of Meds433. These data show that GPT1 downregulation could be one of the possible mechanisms in which Meds433 acts in CML. **Conclusions:** Our study shows that DHODH inhibition is a promising approach for targeting CML stem/progenitor cells and may help more patients discontinue the therapy. This work was supported by Associazione Italiana per la Ricerca sul Cancro: IG-23344 **Keywords:** CML, chronic myeloid leukemia (CML), dihydroorotate dehydrogenase, Meds433

CML-184

A Novel Droplet Digital PCR Strategy for Rapid and Sensitive Detection of BCR::ABL1 Kinase Domain Mutations Conferring Resistance to Second-Generation Tyrosine Kinase Inhibitors

Sara De Santis PhD[1], Cecilia Monaldi PhD[1], Margherita Martelli PhD[1], Manuela Mancini PhD[2], Samantha Bruno PhD[1], Fausto Castagnetti MD[1], Gabriele Gugliotta MD[1], Katerina Machova Polakova PhD[3], Thomas Ernst MD[4], Dianna Maar PhD[5], Adam Corner PhD[6], Michele Cavo MD[1,2], Simona Soverini MD[1]

[1]Department of Experimental, Diagnostic and Specialty Medicine (DIMES), Institute of Hematology "L. e A. Seràgnoli", University of Bologna, Bologna, Italy. [2]IRCCS Azienda Ospedaliero-Universitaria di Bologna, Istituto di Ematologia "Seràgnoli", Bologna, Italy. [3]Institute of Hematology and Blood Transfusion, Prague, Czech Republic. [4]Abteilung Hämatologie/Onkologie, Klinik für Innere Medizin II, Universitätsklinikum Jena, Jena, Germany. [5]Bio-Rad Laboratories, Digital Biology Group, Pleasanton, USA. [6]Bio-Rad Laboratories, Digital Biology Group, Peterborough, United Kingdom

Context: Testing for BCR-ABL1 kinase domain (KD) mutations should always be performed before tyrosine kinase inhibitor (TKI) changes. Next-generation sequencing (NGS) is the best approach to highlight emerging mutations in patients not responding adequately to TKI therapy. However, NGS requires sample centralization and batch analysis and has a non-negligible time to results. In this study, we set up and validated a novel droplet digital PCR (ddPCR)-based multiplex strategy for the detection and quantitation of transcripts harboring mutations impacting TKI selection. **Methods:** In collaboration with Bio-Rad, a 3-tube ddPCR strategy was designed that enables identification and quantitation of 16 nucleotide substitutions encoding the 13 mutations associated with resistance to one or more second-generation TKI (2GTKI). Primers and FAM- or FAM/HEX-labelled probes were grouped on a TKI-specific basis and generated clusters of droplets mapping to spatially distinct areas of the 2D plot based on resistance profiles. Each tube also incorporated primers and HEX-labelled probes for e13a2, e14a2, and e1a2 BCR::ABL1 fusion transcripts to express results as the percentage of mutation-positive over total BCR::ABL1 transcripts. For validation, a total of 101 RNA samples from healthy donors, TKI-sensitive and -resistant patients, and BCR::ABL1-positive and -negative cell lines were used. cDNA (125 ng) obtained with ABL1-specific primers was analyzed in duplicate on a QX200 ddPCR system (Bio-Rad). **Results:** The limit of blank was determined using 60 blank samples. Accuracy and specificity were confirmed using 48 samples positive for one or more 2GTKI-resistant mutations or mutations at nearby codons (to exclude cross-reactivity). Analysis of serial dilutions of cell line mixtures made using BCR::ABL1-positive mutation-positive cells, BCR::ABL1-positive unmutated cells, and BCR::ABL1-negative cells to mimic different mutation frequencies (70%, 5%, and 0.5%) and different transcript levels (MR0 to MR3) showed that a 0.5% lower detection limit could be consistently