EP728 CHARACTERIZATION OF PHENOTYPIC AND GENOTYPIC MARKERS AS PREDICTORS OF RELAPSE DURING TREATMENT-FREE REMISSION IN PATIENTS WITH CHRONIC MYELOID LEUKEMIA L. Vigon1 , M. Galan1 , A. Luna2*, S. Rodriguez-Mora1 , G. Bautista3 , *J. L. Steegmann4* , M. Piris-Villaespesa2 , M. R. López-Huertas1 , *V. Garcia-Gutierrez2* , M. Coiras1 1 Immunopathology Unit, Carlos III Institute, 2 Hematology, Ramón y Cajal Hospital, 3 Hematology, Puerta de Hierro Hospital, 4 Hematology, La Princesa Hospital, Madrid, Spain

Background: Chronic myeloid leukemia (CML) is caused by spontaneous generation of a mutated tyrosine kinase with constitutive activation (BCR-ABL). After several years of treatment with tyrosine kinase inhibitors (TKIs), patients with sustained, deep molecular response (DMR) may interrupt treatment but approximately 50% experience relapse at some point after withdrawal. In addition to their direct effect on the BCR-ABL+ cancerous clone, TKIs are also immunomodulatory drugs that induce a potent antileukemic, cytotoxic response during treatment. It is unknown why this response, based on Natural Killer (NK) and CD8+ T cells, is only conserved in some patients after discontinuation, whereas others relapse and have to restart treatment. Aims: To analyze phenotypic and genotypic markers that may be used as predictors of relapse in patients with CML during treatment-free remission (TFR).

Methods: We recruited 93 samples for analysis divided in 5 groups. “On-TKI”: 45 patients with CML on treatment with TKIs for at least 9 months (imatinib (11), nilotinib (9), dasatinib (20), bosutinib (5)); “OffTKI”: 17 patients on successful TFR for at least 7 months; “Relapse”: 7 patients who relapsed during TFR: 3 samples prior to TKI reintroduction and 4 samples who had already restarted TKIs; “New diagnosis”: 4 patients still untreated with recent CML diagnosis; and 20 healthy donors as basal controls. PBMCs were subjected to phenotypic analysis by flow cytometry. Genotyping of HLA-E and KIR genes was performed using real-time qPCR.

Table 1. Phenotypic analysis in different groups of patients with CML. Leukocytes counts displayed in %.

	CD56+	CD3-CD56+CD16+	CD8+TCRγδ+	CD8-TCRγδ+	CD86+
Healthy donors	10,4±0,6	11±1,1	5,5±0,7	5,7±0,9	5,3±0,5
On TKI	19±1,4	12,9±1,7	11,8±1,2	6,5±0,9	13,4±1,2
Off TKI	13,8±1,7	19,1±3,9	24,5±4,6	11,8±1,9	10,9±1,2
Relapse	4,3±0,3	7,1±0,1	6,5±3,3	4,0±,2,0	20,1±0,2
On TKI after relapse	2,9±1,1	25,9±6,8	9,7±5,0	4,3±2,1	1,7±0,2
New CML diagnosis	6±04	2,4±0,5	4,1±1,3	3,3±1,0	14,2±6,5

Results: 1) Patients’ results 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 relapse. This cell population was not recovered in these patients 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 TFR, regarding patients “On TKI”. This population was reduced 12±3.8% in patients “Off TKI” who relapsed but increased 18.8±6.7% once treatment was restarted. 4) 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. 5) Analysis of HLA-E alleles showed that 60% of patients “Off TKI” were heterozygous for HLA-E, whereas only 15.4% of patients on relapse

were heterozygous, being predominant (61.5%) homozygous HLA-E*0103/0103 genotype in this group. 7) KIR genes encoding for inhibitory molecules KIR2DL2 and KIR2DL5 and activating molecules KIR2DS2 and KIR2DS3 were present in > 71% of patients who relapsed after treatment interruption, regarding < 50% in “Off TKI”. Consequently, 86% of patients who relapsed showed KIR haplotypes BX. Summary/Conclusion: We identified several biomarkers as potential predictors of relapse in CML patients during TFR: CD56+ < 4%; CD3-CD56+CD16+ < 7%; CD8+TCRgd+ < 7%; CD8-TCRgd+ < 4%; CD86+ > 20%; homozygosis for HLA-E*0103; and KIR haplotypes BX. These biomarkers need to be validated in a larger, longitudinal cohort of patients.