Abs N. 0001 (1 of 7)
MYELODYSPLASTIC SYNDROMES - BIOLOGY

AZACYTIDINE AND VENETOCLAX EFFECT ON PHOSPHOLIPASES C AND APOPTOTIC PATHWAY IN MYELODYSPLASTIC SYNDROMES (MDS)

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Introduction: Myelodysplastic Syndromes (MDS) are currently treated with Azacytidine (AZA), alone or in combination with Venetoclax (VEN) (Bazinet A et al. Curr Treat Options Oncol 2022). Phospholipase C (PLC) beta1 has been identified as an azacytidine direct target, and it is associated with a favorable response in higher-risk MDS (Cocco L et al. J Leuk Biol 2015), where it can regulate apoptotic pathways. Here, we further investigated the molecular effect of AZA and AZA+VEN in high-risk MDS patients and hematopoietic cell lines.
Methods: Mononuclear cells, isolated from 8 higher-risk MDS patients (RAEB-1, R-IPSS High or Very High, Complex Karyotype), treated with AZA (n=5) and AZA+VEN (n=3) at the IRCCS-Institute of Hematology "L e A Seràgnoli", Bologna, were used to carry out molecular analyses at diagnosis and during therapy. THP-1 and MV4-11 leukemic cells, used as resistant and sensitive in vitro models, were treated with AZA/AZA+VEN and, after 24 hours, flow cytometric, molecular and Western Blot analyses were performed.
Results: In 2/5 AZA-responder patients (R), apoptotic markers were modulated within the first cycles of therapy (BCL-2 decreasing, BAX increasing), while, in AZA non-responders (NR, 3/5), BCL-2 increased, and BAX decreased. All 3 AZA+VEN patients showed a rapid hematological response, an early BCL-2 decrease and a BAX increase. However, one AZA+VEN R patient, which subsequently evolved into Acute Myeloid Leukemia (AML), showed a BCL-2 increase and no significant changes in BAX.
In both cell lines, AZA+VEN strongly and rapidly increased in vitro cell death, and this was partially confirmed by apoptotic markers expression. Even Western Blot analyses showed an activation of apoptosis, via caspase 3 and the activated form of PARP, i.e., one of its downstream targets. Moreover, AZA+VEN, but not AZA alone, significantly decreased the G2-M phase in both cell lines and early induced PLCbeta1, although only in THP-1 cells, since PLCbeta1 decreased in MV4-11 cells.
Conclusions: Ex vivo analysis of MDS patients treated with AZA+VEN revealed an increase in PLCbeta1 expression and the pro-apoptotic gene BAX. Even in vitro analyses showed a significant increase in apoptotic cell death following AZA+VEN treatment, and this was partially confirmed by both apoptotic marker expression and the involvement of caspase activity. Even changes in cell cycle were observed, particularly in Venetoclax-resistant cells, leading to G0-G1 phase arrest. Finally, AZA+VEN treatment affected PLCbeta1 expression, an enzyme related to G0/G1 regulation and myeloid differentiation markers. All in all, our results may pave the way to new molecular mechanisms involving PLCs and apoptosis regulation in MDS response to AZA/AZA+VEN, possibly leading to new therapeutic targets.

Pubblicabile: YES, publishable

Abs N. 0002 (2 of 7)
MULTIPLE MYELOMA – CLINICAL

THE ROLE OF RADIOLOGICAL RESEARCH METHODS FOR DIAGNOSING THE PROGRESSION OF MONOCLONAL GAMMOPATHY OF UNDETERMINED SIGNIFICANCE

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Introduction: Monoclonal gammopathy of undetermined significance (MGUS) is a precancerous disease characterized by less than 10% plasma cell infiltration in the bone marrow, absence of symptoms and organ lesion with a 1% risk of progression per year. Detection of skeletal lesions in MGUS during follow-up is one of the signs of progression. The use of x-ray methods of research (low-dose CT) and MRI (diffuse-weighted) conducted in dynamics can help identify patients at an early stage of multiple myeloma.

Materials and Methods: The study included 148 patients with MGUS in Gomel region, Belarus The observation period was 40 months. All patients underwent aspiration biopsy, biopsy of the iliac wing with histological and IHC examination of the bone marrow (BM). Imaging studies included low-dose whole-body CT and diffusion-weighted whole-body MRI performed at intervals of 1 time per year.

Results: The median age in patients with MGUS was 62.0 years (54.0 and 67.0), among the patients, females predominated (62.8%). During the initial examination of patients, the lesion of the skeletal bones was represented by areas of diffuse infiltration of the bone marrow (according to DVMRI), without foci of osteodestructive lesions.

According to IHC data, in this group of patients, an interstitial or diffuse type of bone marrow infiltration with large accumulations was detected at the time of initial diagnosis.

Progression from MGUS to MM was determined in 8.8 % (13) of cases during observation period. Time to progression ranged from 3 to 36 months. Progression in the form of detection of foci of destructive lesions was detected in 6 (46.2%) of this group. These patients had no other signs of progression to symptomatic MM. Lesions of the skeletal bones were represented by the appearance of foci of destructive lesions in the ribs, spine and pelvic bones.

Conclusion: Due to the dynamic observation of patients using low-dose CT and MRI (diffuse-weighted) of the whole body, we were able to identify patients at an early stage of the process, which made it possible to prevent severe complications by prescribing specific therapy.

Pubblicabile: YES, publishable

Abs N. 0003 (3 of 7)
ACUTE LYMPHOCYTIC LEUKEMIA – CLINICAL

A RARE CASE OF CONGENITAL LEUKEMIA WITH LEUKEMIA CUTIS

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Background and aims: Congenital leukemia (CL) is an extremely rare childhood malignancy whose clinical signs develop within the first 28 days of life. CL represents approximately 0.8% of all childhood leukemias and occurs in 1 of 1-5 million births. CL is almost always lethal without chemotherapy, and with chemotherapy, 2-year survival rate may be expected to be 23%. The abandonment rate in low- and middle-income countries (LMIC) for childhood lymphoblastic leukemias is 29%.
Materials and methods: This is a case report of an 8-days-old newborn with CL with leukemia cutis, which led to an early diagnosis of the disease.
Results: A 4-days-old newborn girl presented with a lesion (leukemia cutis?) of the upper third of the right forearm (2.21x0.83cm) to the pediatric department of the Muratsan Hospital Complex. At presentation, the patient had hyperleukocytosis (WBC-89.62x109/L) with 70% blast cells in the peripheral blood and 54% blast cells in the bone marrow. Immunophenotyping revealed the blast population (67%) is mainly B lymphoid (CD19-96%, CD34-43%, CD10-25%, CD38-99%, HLA-DR-85%, TdT-44%, CD79a-95%, CD22-14%). Molecular cytogenetics (FISH) was performed, which revealed homozygous deletion of the p16 (CDKN2A) gene and rearrangement of the MLL (KMT2A) gene. We planned to transfer the patient to the Pediatric Cancer and Blood Disorders Center of Armenia for further chemotherapy. Unfortunately, the parents refused further treatment and took the child home, whereupon the child died 5 days later. On discharge, WBC was 382.12x109/L.

Conclusions: Abandonment of treatment is a major cause of treatment failure and poor survival in children with Childhood Leukemia in LMIC. Treatment outcomes could be substantially improved by strategies that help prevent abandonment of therapy. Reducing treatment abandonment of CL in LMIC requires not only public awareness about leukemias, but also parental education, counseling and psychosocial support during therapy, improvement of quality-of care and adequate management of side-effects.

Pubblicabile: YES, publishable

Abs N. 0004 (4 of 7)
CHRONIC MYELOID LEUKEMIA – BIOLOGY

EFFECTS OF ANTI-SARS COV2-2 MRNA VACCINATION IN CHRONIC MYELOID LEUKEMIA (CML) PATIENTS ACCORDING TO THE TYPE OF TYROSINE KINASE INHIBITORS AND MOLECULAR RESPONSE

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Introduction To date, before Covid-19 vaccination campaign on 27 December 2020, few studies have examined the impact of first- and second-generation Tyrosine Kinase Inhibitors (TKIs) on B-cell responses to vaccination in patients with CML. Treatment with TKIs has been associated with loss of memory B-cell subsets and impaired humoral immune responses to PPS and influenza vaccine, likely driven by the off-target kinase inhibitory activity of these drugs. However CML pts demonstrated to have a good serological response to anti-SARS-COV-2 mRNA vaccination (BNT162b2).

Methods We aimed to study the impact of TKIs and molecular response on the in vivo B-cell response to COVID-19 vaccination in terms of serological response. We studied serological response to BNT162b2 in 44 CML pts (median age 63,19-88) after 30-45 days post first cycle of vaccination and post administration of 3rd dose. 25 pts were treated with Imatinib while 19 received 2nd and 3rd generation TKIs. 6 pts were in MR2 while the others had reached a MR3 or a deep molecular response (DMR). A comparison with 33 healthy health workers of San Carlo Hospital in Potenza (median age 40, 28-66) was foreseen.

Results In the control group mean titer of anti-SARS-CoV-2 IgG was 11876 AU/mL (range 171-40000), in our cohort of CML pts we observed an IgG titer not significantly different compared with the control arm (mean 8700,5, range 105–27951). Fig. 1-A. We observed levels of IgG greater in pts who had received 2nd and 3rd generation TKI than in pts who had used Imatinib (1130,8 vs 6532,7), this data was confirmed also after 3rd dose. Fig. 1-B. Although this trend was not statistically significant, it could be influenced by different median age between two groups of pts (Imatinib 66 vs 2nd and 3rd generation TKI 57 years) and it should be confirmed on a larger sample size. Pts who had achieved at maximum MR3 showed IgG titers greater than pts in DMR (13326 vs 6926) regardless of the TKIs but it warrants further studies on large scale. Fig. 1-C. We also evaluated serological response after 3rd dose in 36 pts, 34 of them showed a further increase of antiSARS-CoV-2 IgG titer. In CML pts mean concentration after 3rd dose was 22179, mean increase compared to the titer after the 2nd dose, expressed as the ratio between the two values, was 3,5 while in the control group the mean titer of IgG after 3rd dose of vaccine was 24443,5 (5756,5–40000) with a mean increase ratio of 2,6.

Conclusions Our study confirmed a good serological response to BNT162b2 for CML pts and this laboratory data was also confirmed by clinical observation of CML pts with COVID-19, all of whom recovered without the need for special care. Differences of serological response observed could correlate with the type of TKIs and molecular response and they would require further studies on

large scale.

Pubblicabile: YES, publishable

Abs N. 0005 (5 of 7)
VIRUS, INFIAMMAZIONE E CANCRO - CLINICA

HUMAN HERPESVIRUS-8 NEGATIVE IN PATIENT WITH NON MULTICENTRIC CASTLEMAN DISEASE AS THE FIRST MANIFESTATION OF HIV DISEASE.

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Background: Patients with acquired immunodeficiency syndrome HIV disease can present with a constellation of symptoms and syndromes. In this abstract we describe a rare case of human herpesvirus 8 (HHV-8) apparently unrelated and not multicentric Castleman disease (CD), as the presenting manifestation of HIV disease. In the context of HIV infection CD is well described and it is almost always multicentric and linked to HHV-8. There are limited published data surrounding HHV-8–unrelated CD among HIV-positive and -negative patients.

Case: We present a case of young 24-year-old boy infected with HIV at first detection with multiple lymphadenopathy whose histology describes a Castelman like picture with negative HHV 8 serology. We have come to our attention this young Brazilian born but adopted by an Italian family at the age of about 6 years. At the onset for which he presented to the ED was fever, asthenia and multiple lymphadenopathies. Laboratory tests: initial lymphopenia, inflammation indices poorly represented, serology for major viruses (CMV-EBV, HBV-HCV-syphilis-quantiferon-toxoplasma-parvovirus) negative. Abdominal lymphadenopathy and splenomegaly were present on the CT scan of the abdomen. Leishmania is also positive for serology and IL-6 2.7pg/mL. A positive HIV test was performed to complete the serum-virological picture. Furthermore, the values of CD4 193/mmc and HIV-RNA 4.730.500 cp/ml are reported, the genotyping test showed a pharmacological pattern of totisensitivity and the avidity for HIV was high precisely to document an acquisition of the disease for several months. He underwent lymph node exeresis whose histology described a picture compatible with Castelman-like. HHV 8 serology was negative. The patient was started on antiretroviral therapy and for the moment on haematological monitoring.

Conclusion: A subset of multicentric CD (MCD) is caused by human herpesvirus-8,whereas HHV-8–negative MCD cases remain idiopathic (iMCD) (2). Unicentric CD (UCD) involves a single lymph node region showing characteristic “Castleman-like” histopathologic changes (1-2). Inflammatory manifestations are generally mild in UCD and usually disappear after surgical excision of the lymph node (1). HHV-8–associated MCD is most commonly diagnosed in HIV-infected or otherwise immunocompromised individuals. However, >50% of MCD cases are HIV and HHV-8 negative (3).The etiology of iMCD is unknown, although it is hypothesized to involve one or more of the following mechanisms: autoimmunity/autoinflammation, paraneoplastic or infection with a virus other than HHV-8 (4).

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Pubblicabile: SI, pubblicabile

AGGRESSIVE SYSTEMIC MASTOCYTOSIS WITH THE CO-OCCURRENCE OF *PRKG2::PDGFRB*, *KAT6A::NCOA2*, AND *RXRA::NOTCH1* FUSION TRANSCRIPTS AND A HETEROZYGOUS *RUNX1* FRAMESHIFT MUTATION

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Introduction: Systemic mastocytosis (SM) is a myeloproliferative neoplasm displaying abnormal mast cell proliferation. It is subdivided into different forms, including aggressive systemic mastocytosis (ASM) and systemic mastocytosis with an associated hematologic neoplasm. Oncogenic alterations include point mutations, mainly the KIT D816V and and JAK2 V617F, conferring poor prognosis and therapy resistanc; and fusion genes, with those involving *PDGFRA/PDGRFB* as the most recurrent events.

Methods: We here describe an SM case, rapidly evolved towards SM associated with acute myeloid leukemia (SM-AML). Cytogenetic (karyotyping and fluorescence in situ hybridization (FISH) assays) and molecular (chromosome microarray analysis, DNA targeted-deep sequencing, RNA-Seq, RT-PCR, and Sanger sequencing) analyses were performed on patient bone marrow (BM)/peripheral blood samples collected from the diagnosis to the SM-AML evolution and patient death.

Results: The patient at diagnosis was negative to the KIT D816V and JAK2 V617F alterations but showed a *RUNX1* frameshift heterozygous mutation (Variant Allele Frequency: 11.4%) and the co-occurrence of three fusion transcripts. The first one, *PDGRFB::PRKG2*, was generated by a balanced t(4;5)(q24;q32) translocation as the sole abnormality, identified by karyotyping the BM cells. Other two novel chimeras, *KAT6A::NCOA2* and *RXRA::NOTCH1*, detected by RNA-Seq analysis and validated by RT-PCR/Sanger sequencing, probably originated from cryptic intra-chromosomal abnormalities, since neither karyotype or FISH analyses detected them.
The evolution towards SM-AML was characterized by the occurrence of a complex karyotype showing multiple chromosome losses and gains and the presence of an extra copy of the der(5)t(4;5)(q24;q34) chromosome, leading to the persistence and increased expression of the *PDGRFB::PRKG2* chimera. Moreover, an increase in the *RUNX1* mutation allelic frequency (Variant Allele Frequency: 44%) was reported. Whereas *KAT6A::NCOA2* and *RXRA::NOTCH1* fusions were not detected, at least considering the sensitivity limits of the used techniques.

Conclusions: Here, we analyze an interesting SM case showing multiple fusion transcripts involving relevant genes in hematological malignancies: *PDGRFB::PRKG2*, occurring from patient diagnosis to death and already reported in SM; and *KAT6A::NCOA2 /RXRA::NOTCH1*, detected in

the ASM phase and possibly having a role in the rapid disease evolution towards SM-AML.
The pathogenic *RUNX1* frameshift mutation was also relevant for ASM disease evolution, as it is considered poor-risk alterations in ASM patients.
Overall, our results indicated that the transcriptional landscape and the mutational profile of SM deserve attention to predict the evolution and prognosis of this complex disease, whose classification criteria are still a matter of debate.

Pubblicabile: YES, publishable