FIFTH INTERNATIONAL SYMPOSIUM ON SECONDARY LEUKEMIA AND LEUKEMOGENESIS

HONORARY PRESIDENT: GIUSEPPE LEONE

CONGRESS ORGANIZERS: FRANCESCO LO COCO, LIVIO PAGANO, MARIA TERESA VOSO

ROMA, SEPTEMBER 22-24, 2016

NH Collection Vittorio Veneto Hotel

www.secondaryleukemia.org

Dear Colleague,

we kindly remind you that the 5th International Symposium on "Secondary Leukemia and Leukemogenesis" will be held in Rome on September 22 -24, 2016. This conference has long since become a traditional, well established meeting that gathers together renowned scientists, from all over the globe. In fact, basic science, translational and clinical experts will update the audience on most recent advances related to both laboratory and clinical research in secondary leukemias. In this field, new scenarios have recently emerged and certain mutations frequent in therapy-related leukemias (e.g. TP53), have been identified in patients' bone marrow at the time of the primary malignancy, before the administration of any cytotoxic therapy, indicating a pre-existing minor clone which expands under the selective pressure of cytotoxic treatment. In addition, somatic mutations in genes recurrently involved in acute myeloid leukemia and myelodysplastic syndromes have been identified in the peripheral blood of normal elderly individuals, changing the concept of susceptibility and correlating it not only to germ-line, but also to acquired genetic changes. The "Fifth International Symposium on Secondary Leukemia and Leukemogenesis" will provide a comprehensive update on clinical and biological issues of this changing landscape, hopefully providing new perspectives for future prevention and treatment of this late complication of cancer therapy. The symposium offers a great opportunity for young investigators interested in the field to present their work in dedicated oral and poster session.

We are delighted to welcome you to the Eternal City!

Francesco Lo Coco *Livio Pagano* *Maria Teresa Voso*

HONORARY PRESIDENT

Giuseppe Leone

CONGRESS ORGANIZERS

Francesco Lo Coco Livio Pagano Maria Teresa Voso

SCIENTIFIC SECRETARIAT

Claudia Carissimo Emiliano Fabiani Luana Fianchi Serena Lavorgna

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Therapy-Related Acute Myeloid Leukaemia: Who Really Has It?

R.P. Gale

Haematology Research Centre, Division of Experimental Medicine, Department of Medicine, Imperial College London, London, UK

Most of our knowledge of a relationship between exposure and developing acute myeloid leukemia (AML) comes from epidemiological studies. So it is important to recall epidemiology deals with associations and not with cause and effect. It is also important to recall epidemiological studies typically consider the association between an exposure and an outcome for a cohort, not a person. Even exposures strongly-associated with an increased risk of AML in a cohort do not allow us to impute this exposure as causative in a person with AML. Here we need to rely on a different calculation: *probability of causation* namely, the likelihood a person's exposure caused or contributed to his/her developing AML. This is complicated. For example, what if the exposure did not cause AML because the person would have developed it in the absence of exposure? What if the exposure merely accelerated developing AML or made it worse but did not *cause* it? To accurately estimate whether a person's AML was caused by or contributed to by a prior exposure we need to know many variables. Typically, many of these are unknown to the person tying to estimate causation. If a haematologist considers all of the available data it is sometimes possible to give a *best estimate* value of the likelihood an exposure caused or contributed to a person developing AML. However, such best estimate values are uncertain and should be accompanied by a confidence or credibility interval indicating a range of possible values. And it is always important to remembers *statistical* inference is only a part of causal inference.

Mutational Landscape Of Therapy-Related Versus Other Secondary Leukemias

M.J. Walter

Washington University School of Medicine, Saint Louis, Missouri, USA

A common set of genes are somatically mutated in patients with myelodysplastic syndromes (MDS), acute myeloid leukemia arising from an antecedent MDS (secondary AML), and therapy-related myeloid neoplasms (t-MN). However, the frequency of mutations is different in these myeloid cancers suggesting that unique mechanisms may drive disease pathogenesis. For example, our group showed that TP53 mutant cells expand following cytotoxic chemotherapy exposure, providing rationale for why *TP53* mutations are more common in t-MN. Consistent with this observation, we were able to detect extremely rare TP53 mutant hematopoietic cells prior to chemotherapy exposure and years before t-MN development. Collectively, the data indicate that selection and expansion of rare pre-existing mutant cells can occur in t-MN patients. We next asked whether a similar finding occurred during disease progression from MDS to secondary AML. Progression is typically characterized by the emergence or expansion of a subclone that is not always detectable at MDS diagnosis. We used an ultra-sensitive molecular barcode-sequencing assay to interrogate paired patient samples where a rising secondary AML subclone was not originally detected in the MDS sample using standard high-coverage next-generation sequencing. Using molecular barcode-sequencing, we were able to detect rare mutant cells at MDS diagnosis years before secondary AML progression and observed that treatment (e.g., decitabine) shaped the mutational landscape of cells destined to become the expanding subclone. Ultimately, detecting and monitoring the expansion of rare mutant cells in patients could be used to assess the real-time risk of secondary or therapy-related leukemia development.

Molecular Analysis Of Del(5q) T-Mn, And Identification Of Haploinsufficient Tumor Suppressor Genes On 5q

A. Stoddart[1], J. Wang[1], C. Hu[1], A.A. Fernald[1], E.M. Davis[1], J. Anastasi[2], J. Cheng[2], M.M. Le Beau[1,2]

[1]Department of Medicine and 2Department of Pathology, University of Chicago, Chicago, USA

Background: t-MN with a del(5q) arises after alkylating agent therapy, and is characterized by a complex karyotype, mutations of TP53, and a poor prognosis. We previously showed that haploinsufficiency of more than one gene on 5q contributes to t-MN, and established a mouse model for del(5q) t-MN, whereby transplantation of BM cells with loss of two 5q genes (Egr1+/-, Apcdel/+) transduced with Tp53 shRNAs resulted in AML in 17% of WT recipient mice.

Results: There is growing evidence that perturbations of the BM niche contribute to leukemogenesis. At present, the effect of cytotoxic therapy on the BM niche as well as the HSPCs is not well understood. Using our Mx1-Cre+,Apcdel/+ model, we previously showed that haploinsufficiency of Apc results in a rapidly fatal MDS mediated by loss of Apc in the BM niche, and is accelerated by concordant loss of Egr1 or treatment with ENU. Reduction of Wnt signaling via the canonical pathway using a conditional genetic model (Apcdel/+, Ctnnb1del/+) or pharmacological inhibition of Ctnnb1 (beta-catenin) rescues the MDS phenotype. In mice transplanted with Egr1+/-, Apcdel/+, Tp53 shRNA HSPCs, exposure to ENU strikingly decreased survival (200d vs 508 d) and led to AML or MDS-MLD (82% vs 17%). In contrast, ENU exposure of either donor or recipient led to MDS/AML in only 10% of mice.

Conclusions: These results suggest that (1) concordant haploinsufficiency of EGR1 and APC plays a key role in the pathogenesis of the early stages of del(5q) t-MN; (2) alterations to the niche leading to altered WNT signaling may be an early event in disease initiation, and a therapeutic target; (3) cooperating TP53 mutations are required for progression to AML; and (4) t-MN is promoted by the combined effects of alkylating agent therapy on both the HSPCs and the niche, making t-MN a disease of the “tissue”.

Epidemiology Of T-Mn: An Evolving Scenario

C. Tarella, E. Derenzini, F. Gigli, S. Sammassimo, A. Gueli, S. Ramadan

Haemato-Oncology Division, European Institute of Oncology, Milano, Italy

Background: Myeloid neoplasms developing in patients who have been exposed to cytotoxic agents are collectively classified as therapy-related myeloid neoplasms (t-MN). According to the World Health Organization (WHO), t-MN include therapy-related acute myeloid leukemia (t-AML), myelodysplastic syndrome (t-MDS), and myelodysplastic syndrome/myeloproliferative neoplasms (t-MDS/MPN) (Vardiman et al, Blood 2009). Overall, t-AML is the most challenging form and it accounts for about 7-10% of all AML diagnoses (Ramadan et al, Blood 2013). The reported latency period between exposure to treatment and the development of t-AML is variable, ranging from few months to several years and depends on the type of therapy, dose schedule, and cumulative dose, as well as patient-related factors (Ramadan et al, Haematologica 2012, Ramadan et al, Blood 2013). Recent developments: Cancer therapy has evolved over the past decade with the introduction of new targeted agents, changes in chemotherapy protocols and radiation therapy approaches. The real risks for AML after chemotherapy across different primary malignancies and in the current treatment era are lacking. The most frequent primary tumors in patients with t-AML are lymphomas and solid tumors (Leone et al, Curr Opin Oncol. 2011, Fianchi et al, Br J Med 2015). The trend of t-AML over the years was evaluated in a number of studies. A stable incidence of t-AML over the years has been observed in Denmark (Østgård et al, J Clin Oncol 2015). On the other hand, data from 9 US registries suggested variation in t-AML risks across different tumors with rising standardized incidence ration (SIR) among lymphoma patients (Morton et al, Blood 2013). The use of chemotherapy, particularly if given at high doses, remain the main risk factor for t-MN development. This has been clearly shown in patients undergoing high-dose therapy with autograft (Tarella et al, J Clin Oncol 2011). To further stress this concept, the incidence of t-MN has been markedly lowered in Hodgkin's Lymphoma since the introduction of less cytotoxic treatment programs (Koontz et al, J Clin Oncol 2013, Eichenauer

et al, Blood, 2014). In this view, the progressive advances of molecularly targeted therapies may considerably reduce the need of intensified chemo-radiotherapy along with effective disease control leading to a drop in the risk of t-MN. The low incidence of t-MN documented in the long-term analysis of the original GELA-study on CHOP vs. R-CHOP in aggressive lymphoma is in line with these expectations (Mounier et al, Clin Lymph, Myel & Leuk 2012). Predictive factors for the development of t-AML: Since not all patients exposed to cytotoxic treatment develop leukemia, individual susceptibility has also been suggested. Therefore, genetic and host related factors potentially predisposing to the development of t-AML are currently the subject of intense research. Evolving evidence at the molecular level supports the idea that it may be misleading to label all leukemias that develop in patients with primary tumors as 'therapy-related leukemias' (Gale et al, Leuk Res 2014). The recent observation that somatic TP53 mutations can be detected in hematopoietic stem cells long before exposure to cytotoxic therapy strongly suggests that the presence of pre-existing mutated clones may concur to t-MN onset (Wong et al, Nature 2015). Early cell ageing is another possible predisposing factor for t- MDS/AML. The accelerated cell ageing of hematopoietic cells has been evaluated by our group by assessing the telomere length (TL) in lymphoma patients exposed to variably intensified chemotherapy schedules. Our observations confirm the role of early cell ageing in t-MN development particularly in patients undergoing transplant procedures. Conclusions. The occurrence of t-MN remains a threatening event that may occur in patients with malignancy undergoing chemo-radiotherapy. This complication is particularly detrimental for lymphoma patients. Optimized chemotherapy use, along with the increasing availability of molecularly-targeted agents are expected to a restrain the risk of t-MN. Moreover, understanding molecular defects in the subgroup of patients who developed leukemia will provide a better understanding of mechanisms contributing to the occurrence of multiple malignancies in the same individual.

Therapy-Related Myeloid Neoplasms (T-Mn) In 84 Patients Following Radiation Therapy (Rt) Only

R.A. Larson, N.Khan, R.J. Lin, H.Weiner, M.Green, T.Karrison, M.M. Le Beau

Department of Medicine and the Comprehensive Cancer Center, The University of Chicago, Chicago, IL, USA

Exposure to ionizing radiation is associated with the development of secondary malignancies including t-MNs. We reviewed 84 consecutive cases of t-MN presenting to the University of Chicago between 1972-2015 in patients (pts) who had received RT alone for a prior disease; 23 (27%) diagnosed since 2000. There were 40 females; 44 males. 61 (73%) were Caucasian. 77 (92%) had had a primary solid tumor, 5 a primary hematologic malignancy (4 Hodgkin), and 2 had RT for non-malignant disorders. Prostate and testicular cancers were the predominant primary tumors (35; 42%). Breast (18; 21%) and gynecological cancers (15, 18%, including ovarian, cervical, endometrial, vaginal) were also common. Median age at primary diagnosis was 64 years (interquartile range (IQR), 51-73). Median latency from RT to t-MN was 68 mos (IQR, 30-119 mos). Pts with hematologic malignancies had shorter latency intervals (median, 25 mos; IQR, 18-50) compared to pts with solid tumors (median, 68 mos; IQR, 33-116). 44 pts (52%) had clonal abnormalities of chromosomes 5 and/or 7, 14 had a recurring translocation, 11 other clonal abnormalities, and 15 a normal karyotype. 41 pts first presented with t-MDS, while 43 pts had >20% blasts at diagnosis. Treatments ranged from supportive care only to intensive chemotherapy and subsequent allogeneic transplantation. Only 9 pts currently remain alive: 3 with inv(16), 1 with t(16;16), 1 with t(15;17), 1 with del(20q), and 3 with normal karyotypes. Median survival was 0.9 years (IQR, 0.6-1.2 years). t-MN following RT alone bears striking clinical and cytogenetic similarities to alkylator-associated t-MN with frequent clonal abnormalities of chromosomes 5 and 7, relatively long latency, and poor outcomes even with intensive therapy. However, some pts with recurring translocations or normal karyotypes have a better response to treatment and longer survivals.

Risk Of Leukemia And Cancer In Myeloproliferative Neoplasms

T. Barbui

Foundation for Clinical Research (FROM), Hospital Papa Giovanni XXIII, Bergamo, Italy

Polycythemia vera (PV), essential thrombocythemia (ET), and primary myelofibrosis (PMF) are chronic myeloproliferative neoplasms (MPN) characterized by clonal expansion of an abnormal hematopoietic stem/progenitor cell. Their natural history is characterized by high incidence of thrombo-hemorrhagic complications, transformation in myelofibrosis and acute myeloid leukemia (AML).

Moreover, in recent years, several studies have evidenced that MPN are associated with an increased risk of solid tumors as well; estimates of standardized incidence ratio (SIR) indicated values of 1.2 for ET and 1.4 for PV. However, these are low values and do not allow to conclude that the incidence of non-hematologic cancers is specifically increased in MPNs.

In contrast, the cumulative incidence of AML in MPN, with death as a competing risk, was consistently found elevated in comparison with normal population and may vary between MPN subsets with PMF being the most likely to transform. Predicting which patients are at the highest risk of transformation to AML may provide an opportunity to intervene early during the course of the disease with specific therapeutic modalities. Exposure to certain cytoreductive agents, cytogenetic aberrations and driver/epigenetic mutations has been shown to increase this risk of both survival and blast phase transformation. Current approaches for patients who transform to AML are associated with dismal outcomes and in the future targeted approaches are expected.

Therapy-Related Myeloid Neoplasms In Patients With Lymphoproliferative Diseases

S. Hohaus

Istituto di Ematologia, Università Cattolica S. Cuore, Roma, Italia.

Therapy-related myeloid neoplasms (t-NM) are part of the spectrum of therapy-related complications in patients with lymphoma cured with cytotoxic treatments.

Hodgkin lymphoma (HL) was the first lymphoproliferative disease with a reported risk of t-MN. The introduction of combination chemotherapy in patients with advanced HL is one of the great success stories of medical oncology and hematology. The high cure rates and long survival of young patients however increased the number of patients at risk. In HL, mechlorethamine and procarabazine are the drugs associated with a particularly high-risk for therapy-related MNs, and also for other secondary cancers. These data, among others, contributed to the fact that ABVD has become and is still the standard chemotherapy regimen in HL. Still, patients who relapse may undergo multiple salvage regimens including high-dose therapy and transplantation that will increase the cumulative risk. Tailoring the therapy by risk-adaptation as in PET-guided protocols reduce the number of patients exposed to potentially harmful drugs and will hopefully further reduce the incidence of t-MN in HL. Epidemiological studies are needed to continuously monitor how changes in therapeutic regimens by the introduction of new biological agents will impact on the risk of t-MN. These agents may replace harmful drugs, thus reducing the risk of t-MN. However, one cannot exclude that these drugs might potentiate carcinogenic mechanisms or increase the risk of t-MN by simply prolonging survival of patients previously exposed to cytotoxic drugs associated with t-MN risk.

The introduction of immunomodulatory drugs, in particular monoclonal antibodies has profoundly changed the therapeutic strategies in Non-Hodgkin lymphomas and increased the probability of survival. The addition of Rituximab has not been reported to increase the risk of t-MN of the widely used CHOP regimen, that is overall associated with a low t-MN risk. On the other hand, data are accumulating on fludarabine, in particular in

combination with alkylating drugs and rituximab, which is listed among the drugs with an intermediate t-MN risk profile. Recently, a crude rate of 5.1% of t-MN during a follow-up period of 4.4 years has been reported in patients with CLL following first-line treatment with FCR. The use of bendamustine is rapidly increasing in low-grade lymphomas and CLL. Bendamustine has a nucleoside moiety similar to fludarabine. Although there are only few cases of t-MN reported following treatment with bendamustine, observational studies are warranted.

Prolonged cytopenias and immune suppression are potential side effects of nucleoside analogues. Stress hematopoiesis during prolonged cytopenias may favour the emergence of genetically or epigenetically altered clones. Changes in immune checkpoints favouring the emergence of malignant clones is an area of intense study in carcinogenesis, but the potential contribution to the emergence of malignant hematopoietic clones leading to t-MN is not well understood.

In conclusion, the development of t-MN remains a concern also in modern lymphoma treatment and careful assessment of therapeutic regimens for their t-MN risk is still warranted.

Mitoxantrone

S.K.Hasan

Mumbay, india

Mitoxantrone is an anthracenedione that differs from the anthracyclines in lacking the sugar and the tetracyclic ring. It is a synthetic drug with three planar rings that intercalates into DNA and inhibits topoisomerase II. Mitoxantrone has been used in chemotherapy regimens for leukemia, lymphoma, breast and prostate cancer, and to treat multiple sclerosis. Mitoxantrone is approved by the US Food and Drug Administration in 1999 for the management of patients with secondary progressive MS without superimposed relapses. Therapy-related acute leukemia (TRAL) is a major apprehension, when considering treatment of multiple sclerosis (MS) with mitoxantrone. Although individual cases of TRAL have been reported even after a single dose of mitoxantrone (12mg/m2) but most of the documented TRAL occurred in individuals exposed to > 60 mg/m2.The risk of TRAL at any dose of mitoxantrone is 0.8%, compared with 0.003% for developing AML in the general population representing a significantly increased relative risk. The median latency between the first dose of mitoxantrone and development of TRAL is 22 months (range 4 to 76 months). Given the latency between exposure to mitoxantrone and onset of TRAL, it is essential that hematological monitoring be continued well beyond active treatment. Several studies report on the proposed pathogenesis of TRAL in particular t-APL identifying a distinct distribution of chromosome 15 breakpoints as compared to de novo APL. It has also been reported that an increased susceptibility to developing t-APL in mitoxantrone-treated MS patients may be related to genetic variants in DNA repair and drug metabolism. While making therapeutic decisions in any disease, it is essential to understand the risk-benefit ratio of any individual therapy. Mitoxantrone has now been replaced by newer immunosuppressive drugs such as natalizumab, alemtuzumab and fingolimod in the developed countries for the treatment of MS.

Radiation And Leukaemia: Atomic Bombs And Other Exposures Including Nuclear Power Facility Accidents

R.P. Gale

Haematology Research Centre, Division of Experimental Medicine, Department of Medicine, Imperial College London, London, UK

Radiation is the strongest, best studied and best understood cause of leukaemia. Data from the 123,00 subjects in the Lifespan Study of A-bomb survivors (94,000 exposed, 29,000 controls, 600,000 person-years) is the best investigated data source. In analyzing these data it is important to understand that only 20% of energy released from the A-bombs was in the form of radiation that exposures were acute, high-dose and dose-rate high-energy γ-photons. Median dose of exposed persons was 200 milliSieverts (mSv range, 0-5 Sv). Increased incidences of acute lymphoid and myeloid leukaemias (ALL and AML) and chronic myeloid leukaemias were seen. There were no significant increases in chronic lymphocytic leukaemia (CLL) or adult T-cell leukaemia lymphoma (ATLL). Latencies to leukaemia development differed with an overall median of about 10 years. However, some radiation-related cases occurred after several decades as did myelodysplastic syndrome (MDS). Leukaemia excess relative risk (ERR) show a linear-quadratic relationship to dose with a fall-off at doses >4-5 Sv. The data are consistent with a no threshold for leukaemia-induction at doses >10-20 mSv. Other data to be discussed include leukaemia risks in nuclear industry workers, persons exposed to ionizing radiations *via* medical procedures, children exposed to terrestrial γ-radiations and populations exposed to ionizing radiations as a consequence of the Chernobyl and Fukushima nuclear power facility accidents. And finally whether CLL is a radiogenic cancer.

Differential Treatment Approaches For Therapy Related Acute Leukemia

A. Venditti

Cattedra di Ematologia, Università "Tor Vergata", Roma, Italia

Treatment of therapy-related acute leukemias (t-AL) still represents a challenge for the hematologists and a field of unmet need for the patients. The reason for the persistent inability to successfully manage these forms of acute leukemias is the resistance to standard chemotherapy, which is conferred by specific genetic and molecular alterations. This observation has fostered the development of alternative agents and strategies such as hypomethylating agents, new formulation of old drugs, new antibody-drug conjugates and molecularly-directed small molecules against specific mutations, commonly occurring in therapy-related ALs. Hypomethylating agents deserve consideration for they have shown to be active in acute myeloid leukemias (AML) with genetic changes resembling t-AL. This was confirmed in a recent large, systematic review of hypomethylating agents trials recruiting *de novo* and secondary AMLs (*Yun et al. Clinical Epigenetics, 2016*). In the line of modification and reformulation of traditional chemotherapy, CPX-351 has gained a lot of attention. CPX-351 is a liposomal formulation of cytarabine and daunorubicin in a 5:1 molar ratio. In a randomized, open-label phase 2 trial, 127 patients age 60 to 75 years with or without secondary AML (AML with a history of an antecedent hematologic disorder or therapy-related AML) were randomly assigned to induction chemotherapy with CPX-351 or daunorubicin/cytarabine. There was no statistically significant difference in terms of CR rate, OS and EFS between the 2 groups. However, a higher CR rate in the CPX-351 group led to a statistically significant 6-month survival benefit in patients with secondary AML.

CO1

Clonal Evolution In Therapy-Related Neoplasms

E. Fabiani [1,2], G. Falconi [1], *, L. Fianchi[1], M. Criscuolo[1], T. Ottone[2], L. Cicconi[2], S. Hohaus[1], S. Sica[1], M. Postorino[2], A. Neri[3], M. Lionetti[3], G. Leone[1], F. Lo-Coco[2], MT. Voso[2]

[1]*Department of Hematology, Universita' Cattolica Sacro Cuore, Rome, Italy*
[2]*Department of Biomedicine and Prevention, Universita' Tor Vergata, Rome, Italy,*
[3]*Department of Clinical Sciences and Community Health, Università degli studi di Milano, Italy*

Topic

Pathogenesis of therapy-related acute myeloid leukemia and myelodysplastic syndromes

Background

Therapy-related myeloid neoplasms (t-MN) may occur as a late effect of cytotoxic therapy for a primary malignancy or autoimmune diseases in susceptible individuals. The aim of our work was to study the development of a panel of somatic mutations in patients who developed t-MN after cytotoxic treatment for a hematologic malignancy.

Material and Methods

We studied 14 patients who developed a secondary leukemia (13 t-MN and 1 t-acute lymphoblastic leukemia, t-ALL) at a median latency of 73 months (range 18-108) from the primary diagnosis of hematologic malignancy. Sanger and next generation sequencing (NGS) were used for screening of somatic mutations in samples collected at the time of t-MN diagnosis, whereas ultra-deep NGS and pyrosequencing methods were used to assess the variant allele frequencies (VAF) of mutations identified in samples collected during the time between primary neoplasm and t-MN onset.

Results

We identified 8 mutations (*ASXL1* Y591*, *ASXL1* S689*, *ASXL1* R69*, *IDH1* R132H, *SRSF2* P95H, *SF3B1* K700E, *SETBP1* G870R and *TP53* Y220C) in seven patients (59%), whereas the t-ALL patient had a t(4,11) translocation. These mutations were then tracked backwards in follow-up marrow samples preceding secondary leukemia occurrence using protocols that allow the detection of very low variant allele frequencies (VAF ≥0.01%). Genetic

alterations were detectable in the BM harvested at the primary diagnosis, prior to any cytotoxic treatment in two patients (figure 1A-B), while they were absent and acquired by the t-MN clone in six patients (figure 1C-H).

Conclusions

These data show that clonal evolution in t-MN is heterogeneous, with some somatic mutations preceding cytotoxic treatment and possibly favoring leukemic development and others probably induced by cytotoxic treatment.

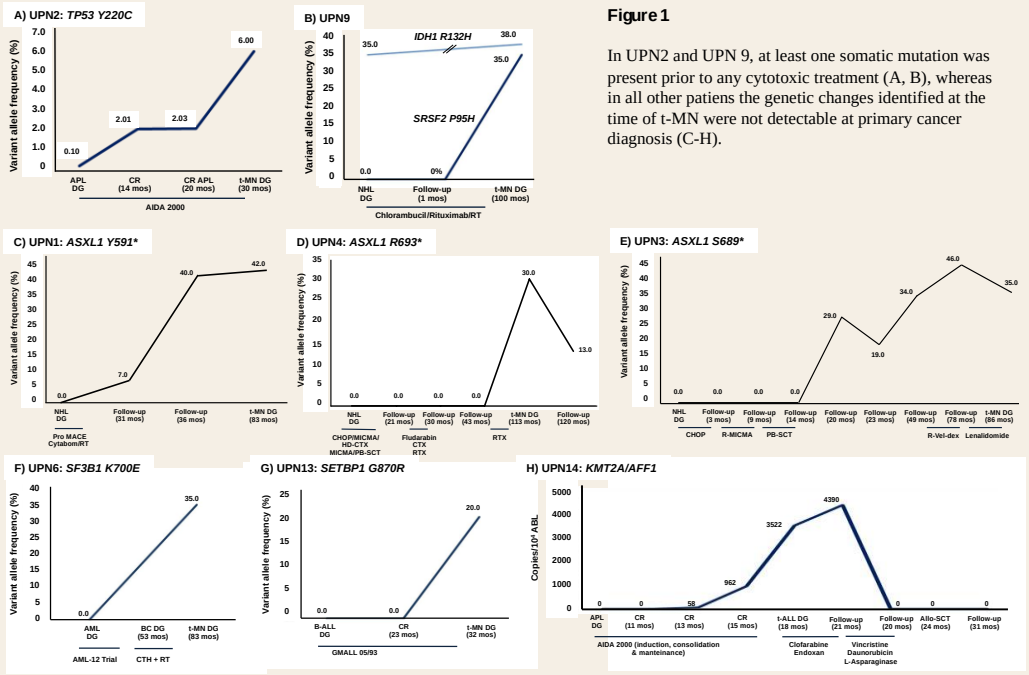


Figure 1

In UPN2 and UPN 9, at least one somatic mutation was present prior to any cytotoxic treatment (A, B), whereas in all other patiens the genetic changes identified at the time of t-MN were not detectable at primary cancer diagnosis (C-H).

Baseline Predictors Of Mortality In Patients With Relapsed Or Refractory Acute Myeloid Leukemia (Aml) Treated With Vosaroxin Plus Cytarabine Or Placebo Plus Cytarabine In The Phase 3 Valor Study

A. Cuneo, MD, PhD[1], J. E. Lancet, MD[2], F. Ravandi, MD[3], E. K. Ritchie, MD[4], H. Sayar, MD[5], S. A. Strickland, MD[6], G. J. Schiller, MD[7], N. Vey, MD[8], H. P. Erba, MD, PhD[9], A. Pigneux, MD[10], H-A. Horst, MD[11], C. Recher, MD[12], O. Odenike, MD[13], A-M. Carella, MD[14], D. Selleslag, MD [15], J. Kell, MD[16], U. Krug, MD[17], L. Page[18], A. DeVault, PhD[18], S. Hwang, MD[18], A. R. Craig, MD, PhD[18], R. K. Stuart, MD[19]

[1]University Hospital Arcispedale S. Anna, University of Ferrara, Ferrara, Italy; [2]Moffitt Cancer Center, University of South Florida, Tampa, FL, USA; [3]University of Texas MD Anderson Cancer Center, Houston, TX, USA; [4]Weill Cornell Medical Center, New York, NY, USA; [5]Indiana University Cancer Center, Indianapolis, IN, USA; [6]Vanderbilt-Ingram Cancer Center, Nashville, TN, USA; [7]University of California, Los Angeles, CA, USA; [8]Institut Paoli-Calmettes, Marseille, France; [9]Division of Hematology and Oncology, University of Alabama, Birmingham, AL, USA; [10]Hôpital Haut Lévêque-CHU de Bordeaux, Pessac Cedex, France; [11]Medizinische Klinik und Poliklinik im Städtischen Krankenhaus, Kiel, Germany; [12]Institut Universitaire du Cancer de Toulouse Oncopole, CHU de Toulouse, Toulouse, France; [13]University of Chicago, Chicago, IL, USA; [14]IRCCS AOU San Martino-IST, Genoa, Italy; [15]AZ St-Jan Brugge AV, Brugge, Belgium; [16]University Hospital of Wales, Cardiff, UK; [17]Medizinische Klinik A, Universitätsklinikum Münster, Münster, Germany; [18]Sunesis Pharmaceuticals, Inc., South San Francisco, CA, USA; [19]Medical University of South Carolina, Charleston, SC, USA

Background: Treatment-related mortality (TRM) scores predict risk of 30-day mortality with intensive treatment in newly diagnosed AML patients (Walter, 2011, *J Clin Oncol* 29:4417-4424). The "simplified" TRM score includes 8 variables: AML type (secondary vs primary), age, performance status, platelet count, serum albumin, WBC count, peripheral blast percentage, and serum creatinine. In the phase 3 VALOR trial in patients with relapsed/refractory AML, 30-day mortality was comparable between treatment arms (7.9% with vosaroxin/cytarabine and 6.6% with placebo/cytarabine). Here, we present a post hoc analysis of 30-day mortality by TRM score in VALOR patients.

Methods: In VALOR, patients (N=711) were randomized 1:1 to receive vosaroxin (90 mg/m^2 cycle 1 [70 mg/m^2 subsequent cycles], days 1, 4) plus cytarabine (1 g/m^2, days 1-5) or placebo plus cytarabine. Simplified TRM

scores were calculated retrospectively according to Walter 2011.

Results: A simplified TRM score could be calculated for 554/705 patients in the VALOR safety population. Median simplified TRM score at baseline was 2.6 (range 0-21) in the vosaroxin/cytarabine arm and 2.6 (range 0-33) in the placebo/cytarabine arm; most patients (473/554, 85%) had a score <8. Patients with lower TRM scores had lower rates of 30-day mortality; secondary vs primary AML was one TRM variable associated with higher mortality rates (odds ratio, 1.51) (**Table**).

Conclusions: The previously validated TRM score for predicting early mortality in newly diagnosed AML also predicted mortality in this relapsed/refractory population. In future studies of vosaroxin (and other intensive regimens), patient selection based upon these predictors of early mortality should be considered.

Table. Thirty-Day Mortality by Simplified TRM Score

	30-Day Mortality, n/N (%)		
Simplified TRM Score	**Pla/Cyt (N=350)**	**Vos/Cyt (N=355)**	**Total (N=705)**
<8	7/228 (3.1)	10/245 (4.1)	17/473 (3.6)
8 to <16	6/31 (19.4)	5/31 (16.1)	11/62 (17.7)
≥16	3/10 (30.0)	5/9 (55.6)	8/19 (42.1)
Missing	7/81 (8.6)	8/70 (11.4)	15/151 (9.9)
Secondary Leukemia			
No	16/284 (5.6)	23/298 (7.7)	39/582 (6.7)
Yes	7/66 (10.6)	5/57 (8.8)	12/123 (9.8)

Cyt, cytarabine; Pla, placebo; TRM, treatment-related mortality; Vos, vosaroxin.

CO3

The Eln Risk Classification Has Prognostic Relevance Also In Elderly Patients With Secondary Acute Myeloid Leukemia And May Support Treatment Decisions. A Retrospective Multicentric Study Of The Rete Ematologica Lombarda (REL)

M. Farina[1], E. Borlenghi[1], C. Pagani[1], C. Basilico[2], M. Bernardi[3], R. Cairoli[4], A.Cortelezzi[5], M. Da Via'[6], A. Ferrario[2], N. Fracchiolla[5], L. Marbello[4], C. Messina[3], A. Santoro[7], E. Todisco[7], M. Turrini[8], P. Zappasodi[6], G. Rossi[1]

[1]Hematology, ASST Spedali Civili, Brescia; [2]Hematology, ASST dei Sette Laghi, Presidio di Varese Osp Circolo Fondazione Macchi, Varese; [3]Hematology and BMT Unit, San Raffaele Scientific Institute, Milan; [4]Hematology, ASST Grande Ospedale Metropolitana Niguarda, Milan; [5]Hematology, IRCSS Ca' Granda Ospedale Maggiore Policlinico, Milan; [6]Hematology, IRCSS Policlinico San Matteo, Pavia; [7]Hematology, Istituto Clinico Humanitas-Cancer Center, Rozzano (Mi); 8Hematology, Ospedale Valduce, Como, Italy

Background: Secondary AML (s-AML) has been considered at hig
h-risk per-se and excluded from ELN-risk score calculation. Allogeneic stem-cell-transplantation (Allo-SCT) is its only curative option. We have analyzed if ELN-risk may be useful to guide treatment decision in elderly s-AML patients who are excluded from Allo-SCT and mainly receive best-supportive-care (BSC) only.

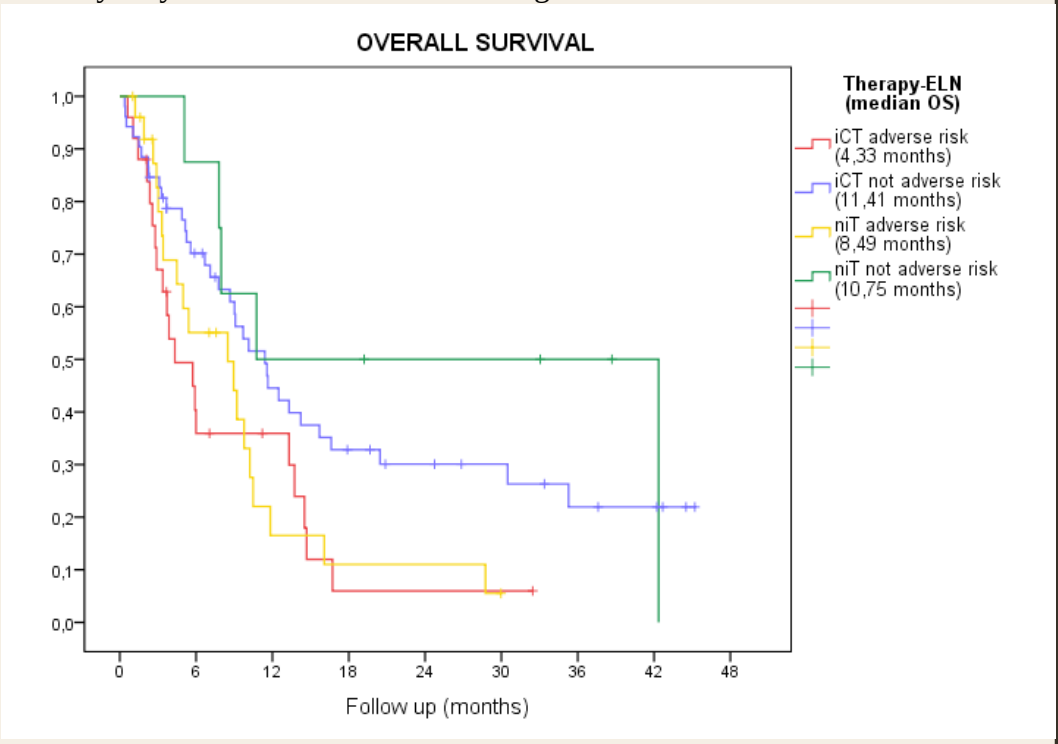
Methods: ELN was applied to 280 s-AML (t-AML 11.4%; post-MDS 60%; post-MPN 28,6%) seen at 8 REL centers; 151 (53.9%) had received intensive therapy (i-T) (34.6%) or non-intensive therapy (ni-T) (19.2%) and 39.1% BSC. Treatment was significantly related to fitness criteria (Ferrara, Leukemia 2013), it was given to 95.4% of non-frail and 4.6% of frail patients.

Results: Outcome was worse with BSC (2.3 vs 9.7 months; p0.000). ELN-risk among 111/151 treated patients was favorable 10.8%, intermediate-I 30.6%, intermediate-II 12.6%, adverse 45.9%.

In treated patients CR achievement (37.7%) was inversely related to ELN-risk (75%, 52.9%, 57.1%, 21.6% in the four categories, respectively; p 0.000). ELN-risk was significantly and independently related to survival (p0.025). In addition also receiving any treatment, but not the treatment intensity (i-T or ni-T), and achieving CR impacted independently on survival in multivariable Cox regression analysis (Figure 1).

Specifically, median survival of fit, non-adverse ELN-risk patients was 11.6 months with i-T, whereas in adverse ELN-risk patients receiving ni-T it was 10.2 months.

Conclusions: ELN-risk calculation is useful also in elderly s-AML. Non-frail patients may benefit from receiving therapy other than BSC, and treatment intensity may be best modulated according to ELN-risk.



Multivariate analysis of prognostic factors for OS*

	HR for OS	P value	IC 95,0% for HR
Therapy i-T-ni-T (n=111) BSC (n=47)	 1 1.822	0.006	1,193 - 2,784
Complete remission yes (n=46) no (n=112)	 0.516 1	0.009	0,314 - 0,846
ELN Class Risk adverse risk (n=83) other (n=75)	 1.76 1	0.006	1,179 - 2,629

* Cox proportional hazards regression model (158 pts with evaluable ELN-risk classification)

CO4

Comparative Genomic Analysis Of *Pml* And *Rara* Breakpoints In Paired Diagnosis/Relapse Samples Of Patients With Acute Promyelocytic Leukemia Treated With Atra And Chemotherapy

L. Iaccarino[1,2]*, T. Ottone[1,2]*, SS K. Hasan[3], M. Divona[1], L. Cicconi[1], S. Lavorgna[1,2], G. Basso[4], E. Such[5], M. Bocchia[6], E. M. Rego[7], D. Grimwade[8], M. T. Voso[1] and F. Lo-Coco[1,2].

[1]Department of Biomedicine and Prevention, Università di Roma "Tor Vergata", Italy.
[2]Laboratory of Neuro-Oncohematology, Fondazione Santa Lucia I.R.C.C.S, Rome, Italy.
[3] Department of Medical Oncology ACTREC, Tata Memorial Centre Navi Mumbai, India.
[4]Department of Women's and Children's Health, University of Padova, Padova, Italy.
[5]Hematology Service, Hospital Universitario y Politécnico La Fe, Valencia, Spain.
[6]Department of Medicine and Immunological Sciences, Division of Hematology and Transplants, University of Siena, Italy.
[7]Department of Internal Medicine, Medical School of Ribeirao Preto and Center for Cell Based Therapy, University of São Paulo, Ribeirao Preto, Brazil.
[8]Department of Medical and Molecular Genetics, King's College London School of Medicine, London, United Kingdom.
*LI and TO contributed equally to this study

Background

It has been reported that t(15;17) occurring in therapy-related APL (t-APL) is frequently generated by cytotoxic drugs targeting the topoisomerase II enzyme. Because standard front-line treatment of APL commonly includes anthracyclines and/or mitoxantrone, we hypothesized that at least in some instances, disease recurrence in APL may be therapy-related, rather than being a true relapse of the original leukemia. While this may occur with no change in the *PML/RARA* isoform, in case of therapy-induced disease, a different genomic breakpoint location could be detectable in *PML* or *RARA* genes at the time of "relapse", possibly with breakpoint been located in one of the "hotspots" *PML* or *RARA* gene regions described in t-APL.

Materials and Methods

We analysed the genomic breakpoints in either *PML* or *RARA* genes in paired samples of 30 APL patients who relapsed after standard ATRA and chemotherapy. In these samples, we verified the potential involvement of the

PML or *RARA* gene "hotspots" typical of t-APL, and/or changes in DNA breakpoints in relapsed samples. Main patient clinical and biological features are shown in Table 1.

Results

PML genomic breakpoints were found to fall between nucleotide positions 1162-1979, within intron 6. None of the *PML* breakpoints was localized within the hotspot region at position 1482-1489, previously identified in t-APL after mitoxantrone treatment. Breakpoints within *PML* exon 6 were localized between nucleotides 661 and 892, whereas in *PML* intron 3 the breakpoints were located between nucleotides 379 and 1326. Concerning the *RARA* gene, breakpoints were scattered between nucleotides 451 and 16477 with no particular clustering. In all cases, we observed identical genomic breakpoint locations in *PML* and *RARA* genes comparing diagnostic and relapse paired samples.

Conclusions

Although APL patients in the present study received the topoisomerase-II inhibitor idarubicin, in addition to mitoxantrone, the molecular profile of the breakpoints at the t(15;17) translocation at relapse was similar to that present at diagnosis, against the hypothesis that some of the relapses were indeed therapy-related APL. A larger series of cases analysed at diagnosis and relapse are needed to confirm this assumption.

Table 1. Main patient clinical and molecular features

UPN	Age/Sex	Sanz Risk*	Treatment	Time of CR (months)	*PML/RARA* isoform	*PML* breakpoint	*RARA* breakpoint
1	55/M	Intermediate	LPA99*	32	bcr1	1594-96	16272-74
2	31/M	Intermediate	LPA99*	45	bcr3	989-91	12158-60
3	34/F	High	LPA99*	19	bcr3	951	16477
4	50/M	Intermediate	LPA 2005*	13	bcr1	1977-79	14447-49
5	39/M	High	IC-APL**	45	bcr3	579	6699
6	38/M	High	IC-APL**	12	bcr3	724	13646
7	29/M	High	IC-APL**	19	bcr3	1285-90	1971-76
8	18/F	High	AIDA 2000*	43	bcr1	1458-60	12324-6
9	NA	NA	MRC*	41	bcr3	855	879
10	NA	NA	MRC*	10	bcr3	819	13299
11	NA	NA	MRC*	12	bcr3	1119-24	14533-37
12	NA	NA	MRC*	14	bcr2	892	14040
13	NA	NA	MRC*	25	bcr2	661	7676
14	46/M	High	AIDA 2000*	26	bcr3	971-2	5394-6
15	42/F	High	AIDA 2000*	9	bcr3	793-4	2285-86
16	42/F	Low	AIDA 2000*	12	bcr3	1050-52	8330-32
17	29/F	Intermediate	AIDA 2000*	NA	bcr1	1356-58	12619-21
18	31/M	Intermediate	AIDA 2000*	12	bcr1	1378-80	9161-63
19	18/F	Intermediate	AIDA 2000*	45	bcr3	912 “A” ins	8889
20	18/F	Intermediate	AIDA 2000*	10	bcr2	758-62	12102-06
21	9/F	High	ICC-APL01***	5	bcr1	1285	13157
22	5/M	NA	AIDA 2000*	20	bcr3	510	642
23	14/M	High	AIDA 2000*	18	bcr3	1323-26	9201-3
24	8/F	High	ICC-APL01***	6	bcr3	1334-5	6897-8
25	58/F	High	AIDA 2000*	19	bcr3	1209-12	7328-31
26	10/F	NA	AIDA 2000*	105	bcr1	1162-5	9932-5
27	10/F	NA	AIDA 2000*	42	bcr3	426-28	451-453
28	45/M	Low	AIDA 2000*	21	bcr1	1649	3341
29	68/F	High	AIDA 2000*	5	bcr3	722	1471
30	56/M	Low	AIDA 2000*	14	bcr3	379 "GA" ins	9295

UPN: unique patient number; CR: complete remission; bcr: breakpoint cluster region; NA: not available; ins: insertion.
*Sanz and Lo-Coco, 2011; **Rego et al, 2013; ***Testi et al, 2014.

CO5

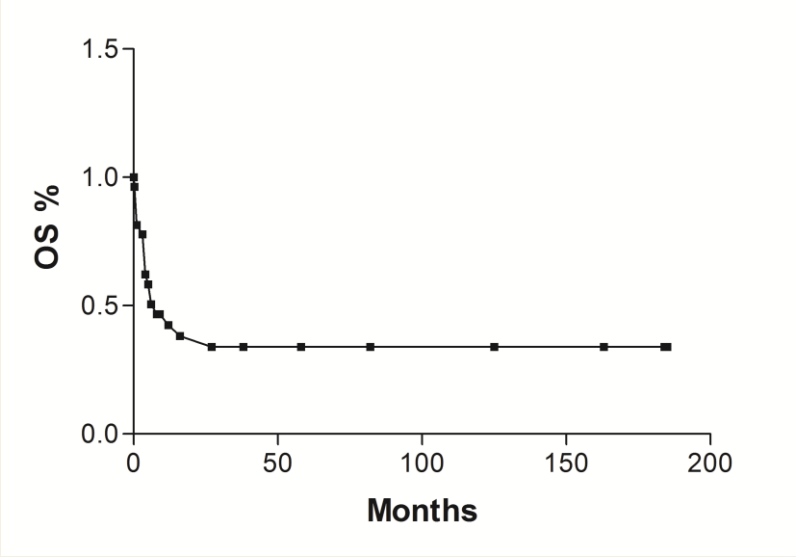
Monocentric Observational Study On Secondary Haematological Neoplasia (t-HN) Submitted To Allogeneic Hematopoietic Stem Cell Transplantation

E. Metafuni, P. Chiusolo, L. Laurenti, F. Sorà, S. Giammarco, S. Sica

Hematology Department, Fondazione Policlinico Agostino Gemelli, Rome, Italy

Background: Therapy related hematological neoplasia (t-HN) occur due to direct mutational events of chemotherapeutic agents and radiotherapy. Disease latency, mutational events and prognosis vary with drugs categories.
Materials and methods: we describe a cohort of 27 patients, 15 females and 12 males, with median age of 53 years (range 29-64), submitted to allogeneic stem cell transplantation (SCT) in our department between September 1999 and July 2016. Patients had a history of solid tumor in 12 cases and hematological disease in 15 cases and all but one received previous treatment. After a median of 36 months (range 12-144) from first neoplasia, patients developed t-AML in 15 cases, t-Ph+ ALL in one case, and t-MDS in 11 cases with karyotype abnormalities in 15 cases. Patients received conventional chemotherapy in 12 cases and 5-azacytidine in 11 cases, while in four patients SCT was performed frontline. Conditioning was MAC in 15 patients and RIC in the others, with graft obtained from related donor in 13 patients and MUD in the others.
Results: fourteen patients obtained sustained CR after SCT, while 8 patients showed resistant or relapsed disease. The remaining five patients died early after SCT. At follow up time (July 2016) 10 patients were alive with a median OS of 70 months (range 3-185), while 17 patients died after a median of 4 months (range 0.3-27) by relapse mortality in 5 cases and non-relapse mortality in the other 12 patients. Figure 1.
Conclusions: Global OS was 37% and after SCT 51.8% of patients with t-HN obtained and maintained CR.

Figure 1. OS of secondary neoplasia after SCT.



CO6

Use Of 5-Azacitidine For Therapy-Related Myeloid Neoplasms In Patients With Concomitant Active Neoplastic Disease

Annereau M[1], Willekens C[2], El Halabi L[2], Chahine C[2], Saada V[3], Auger N[3], Danu A[2], Bermudez E[1], Lazarovici J[2], Ghez D[2], Leary A[4], Pistilli B[4], Lemare F[1], Solary E[2,5], de Botton S[2,5], Desmaris R-P[1] and Micol J-B[2,5].

[1]*Department of Pharmacy, Gustave Roussy Cancer Campus Grand Paris, Villejuif, France*
[2]*Department of Hematology, Gustave Roussy Cancer Campus Grand Paris, Villejuif, France*
[3]*Department of Medical Biology and Pathology, Gustave Roussy Cancer Campus Grand Paris, Villejuif, France*
[4]*Department of Oncology, Gustave Roussy Cancer Campus Grand Paris, Villejuif, France*
[5]*Inserm UMR1170, Gustave Roussy Cancer Campus Grand Paris, Villejuif, France*

Abstract

Background

Patients diagnosed with therapy-related myeloid neoplasms (TRMN) with concomitant active neoplastic disorder (CAND) are usually proposed for best supportive care. The aim of this study was to evaluate the feasibility of using 5-azacytidine (AZA) in this setting.

Methods

All patients referred to Gustave Roussy Cancer Center between January 2010 and December 2015 for TRMN diagnosis and eligible for AZA treatment were included. Patient's outcomes were analyzed based on the presence or not of a CAND.

Results

Forty-seven patients with TRMN were analyzed, including 14 patients (30%) who presented TRMN with CAND. Baseline characteristics were similar in the 2 groups except a trend for best performance status in patients with CAND (p=0.06). AZA dose intensity (median delay between AZA cycles: 29 days [28-32]) was respected in patients with CAND despite association of neoplastic specific treatment in 7 patients. No unusual toxicities were seen in these patients. Overall response rate (71.4% *vs* 60.3%), achievement of transfusion independence (50.0% *vs* 45.5%) and overall survival (12.7 months vs 10.8 months) were similar between patients with CAND and patients without respectively (p=ns). Deaths occurring in patients with CAND were mainly

related to TRMN progression (55%).

Conclusion

Here we report the feasibility and efficacy of AZA for selected patients with TRMN and a concomitant active neoplastic disorder. Interaction between oncologists and hematologists is mandatory to select patients who could benefit from this strategy.

CO7

Survival Improvement in Therapy Related Myeloid Neosplasm ? A Single Center Analysis of 428 Patients.

Saleh K[1], Willekens C[1], Martin J-E[1], Kossai M[1], Khalifé-Saleh N[1], Saada V[1], Auger N[1], Danu A[1], Lazarovici J[1], Leary A[1], Delaloge S[1], Bourhis J-H[1], Solary E[1], Koscielny S[1], de Botton S[1] and Micol J-B[1].

[1]*Gustave Roussy Cancer Center, Villejuif, France.*

Background

t-AML/MDS have a dismal outcome. We wondered if an increase of OS was observed in the last 30 years.

Material and Methods

All patients with a t-AML/MDS diagnosed and/or treated for their prior neoplasm at Gustave Roussy between 1986 and 2016 were included.

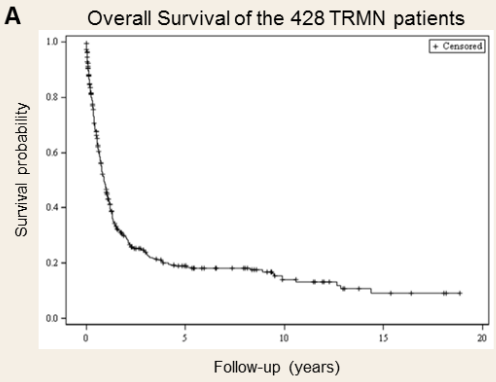
Results

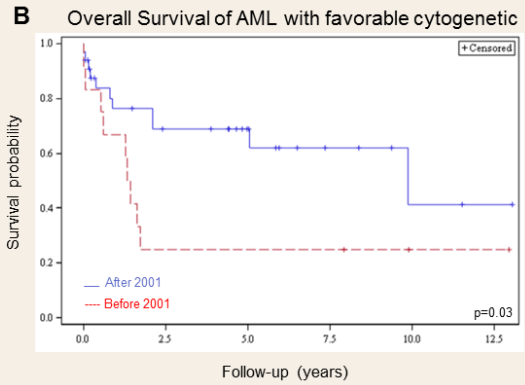
428 patients were analyzed, median age at diagnosis was 56.4 years, 52.3% patients had t-AML. In the t-AML subgroup, 47% of patients presented unfavorable cytogenetic, 26% intermediate and 26% favorable. In the t-MDS subgroup, 78% of patients had an IPPS score Int 2 or High. The median OS was 10.6 months and the 5-year survival was 19.1%. Patients with favorable risk t-AML had better 5-year survival compared to others cytogenetic groups (55.5% vs 20% vs 12.1% respectively, $p<0.001$). Int 2 or High t-MDS was associated with worst 5-year OS compared to others t-MDS (6.6% vs 34.9%, $p<0.001$). We next compared OS after or before July 2001. A trend for better OS for patients diagnosed in the last 15 years was observed (21.5% vs 15.1%, $p=0.39$). Interestingly outcome of t-AML patients with favorable subtype significantly improved over the last 15 years (68.8% vs 25%, $p=0.03$) which was not the case for other cytogenetic subgroups and for t-MDS. Patients who received allograft had a trend for better survival in the last 15 years (52.8% vs 21.5% $p=0.2$).

Conclusions

t-AML/MDS are associated with a low 5-year OS (19.1%) but our results are upper than previous publications. However, a significant improvement of survival in favorable t-AML was observed.

Figure 1


A
Overall Survival of the 428 TRMN patients
+ Censored
Survival probability
1.0
0.8
0.6
0.4
0.2
0.0
0
5
10
15
20
Follow-up (years)


B
Overall Survival of AML with favorable cytogenetic
+ Censored
Survival probability
1.0
0.8
0.6
0.4
0.2
0.0
After 2001
Before 2001
p=0.03
0.0
2.5
5.0
7.5
10.0
12.5
Follow-up (years)