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THE EVOLVING LANDSCAPE OF ACUTE MYELOID LEUKEMIA TREATMENT.

SHORT COMMUNICATION

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Acute Myeloid Leukemia (AML) is a fatal disease. At least two thirds of the patients die of AML in the first few years of diagnosis, and most of them in the first year. [1]

There are several mutations involved in the pathogenesis of this heterogeneous disease and some impact survival; [2] all result in the abnormal functioning of a component of a molecular pathway involved in cell cycle activity or apoptosis. Some of these latter mutations are targetable, and new target drugs are been tested or already used in clinical practice, with objective impact on disease severity and overall survival. [3, 4]

Additionally, there are several new drugs either targeting leukemia microenvironment molecules, or its biophysical aspects, intent to changing the molecular milieu of the malignant cell surroundings; some targeting the malignant cell metabolism or oncoprotein metabolites, and yet some epigenetic drugs aiming to chromatin stabilization and control of malignant genes transcription activity. [5] These drugs might, in selected cases, be utilized in association with one another or to a less toxic and very effective low dose chemotherapy, or yet as monotherapy for the very old and ill population. Complete and partial remission (CR or PR) or stable disease is seen in this scenario and ways to maintain it are been tested and used.

Technics to measure disease burden evolved in the last decades leading to the understanding of measurable residual disease and its impact in AML prognosis. Measurable tumor burden before stem cell transplant (SCT) is alone a risk factor for relapse and disease progression after transplant, usually leading to death in the first few months. [6-7] By regularly measuring residual disease, relapse can be detected in an asymptomatic patient and preemptive therapy can be putted in place. Usually, in the majority of SCT centers, the first clinical intervention is lowering immunosuppression or withdrawing it completely, sometimes followed by donor lymphocyte infusion (DLI), hoping to harness the graft versus leukemia effect (GVL), frequently and unfortunately, accompanied by graft versus host disease (GVHD). However, GVHD is not particularly prevalent or severe after DLI when utilized in the prophylactic setting according to a recent metanalysis. [8]

Emerging data on the above-mentioned target drugs, are increasingly robust, and better quality of live seems to be one outstanding aspect. Side effects of molecular target drugs are mild and manageable.

For many decades, intensive chemotherapy followed by SCT in intermediate and adverse risks disease is the backbone of AML treatment; [9] however, most patients are elderly and dye during or following intensive treatment, [10, 11] since they frequently have comorbidities and develop several complications during the myeloid and lymphoid ablated periods. Most of them are not eligible for stem cell transplantation (SCT), and once AML relapses, as pointed above, survival is very poor.

The understanding of the graft versus leukemia (GVL) effect and its importance for SCT success, [12] as well as the good results obtained with DLI in obtaining CR or disease control (8) have brought into attention the role that immune cells have in leukemia's control and cure.

SCT for AML should be preferentially myeloablative in order to decrease tumor burden in those with high-risk leukemia and good performance status. Myeloablative strategies by killing abnormal and normal leucocytes, modify bone marrow microenvironment. Steven Rosenberg *et al.* [13] have suggested that myeloablative strategies can affect the

general microenvironment that becomes rich in myeloid and lymphoid colony-factors and stimulating molecules.

Pre-clinical and clinical studies with adoptive transfer of lymphocytes have proven beneficial effects in cancer. [14, 15] However, the ***Vito*** effect have to be taken into consideration in immunotherapy. The patients' resident lymphocytes or leukocytes can kill the incoming cells preventing the infused cells *in vivo* activation, either in blood circulation or at the tissue level. According to Rosenberg, myeloablation and/or lympho-ablation is the optimum scenario for adoptive immunotherapy efficacy. [13]

Systemically infused *in vitro* expanded lymphocytes, once into the circulation, can sense the increased concentration of activating molecules, migrate toward the origin of its production and home to that environment, unleashing its anti-tumor and immunomodulatory activity.

The benefic role of CAR-T cells on B cell malignancies is indisputable and much have been learned from it; one key aspect is that the *in vivo* CAR-T cells expansion/activation appears to be related with a better anti-tumor effect. CAR-T cell therapy has also brought into attention cytokine release syndrome (CRS) as well as its unexpected CNS effects, opening a new path to better understanding *in vivo* immune system function, its pros and cons, and how to clinically manage it. [15] The utilization of anti-PD1 receptor or its ligand monoclonal antibodies have proved that exhausted lymphocytes can be reactivated in the tumor microenvironment – making the case for autologous adoptive immunotherapy, with significant results been seen in the solid tumor scenario particularly in lung cancer. [16] Taken together, these mounting data support immunotherapy trials for the treatment of cancer.

Natural killer cells have repeatedly been shown to have antitumor, [17] and antileukemia effect [18-20], and it appears related to GVL effect as its early emergency after SCT myeloablation is correlated with PDS and OS. [21] Since pioneering studies of Velardi's group, [22] in Italy, in a population of AML patients predominantly in second CR and submitted to a T cell depleted haploidentical SCT, GVL's mediated NK cells anti-leukemia effect have been recognized.

NK cells are innate lymphocytes bearing natural cytotoxic receptors that recognize molecular patterns (common to all effector's cells) and several other receptors to ligands on altered cells' surfaces. Killer Immunoglobulin like Receptors [23] were first described in and are predominantly express by NK cells. Their main role is to inhibit NK cell activation, although some are actually able to promote it. These latter are expressed by individuals belonging to NK cell B Haplotype-type, since NK cells from these individuals are characterized by expressing an excess of KIR activating receptors.

Been able to recognize one's self HLA class I or a normally expressed HLA class I antigen, renders NK cells disabled to kill a normal cell. In summary, NK cell activation, either to develop cytotoxicity or secrete immune molecules, is the result of balancing the amount and activation of activator and inhibitory receptors.

NK cells are CD3 negative and CD56 positive cells. Most of our peripheral blood circulating NK cells also express CD16, a FC receptor to immunoglobulin that promotes ADCC. For many years $CD56^{+}CD16^{+++}$ NK cells were considered the NK cell mature, functional phenotype. [24] However, tissue resident or occasional tissue transiting NK cells express a variable, apparently tissue dependent phenotype, in the lung, as an example 75% of tissue resident NK cells are $CD56^{bright}$ with variable low or no expression of CD16. [25, 26]

Innate lymphocytes are meant to bridging innate and adaptive immunity. [27] As for NK cells, it has been shown they modulate T cell response either by IFN-gamma or through GM-CSF secretion since it has an important role in promoting T cells and Dendritic Cells (DC) maturation and activation, and in the case of DC, also migration and antigen presentation untimely promoting adaptive immune reaction. [28]

It is possible that NK cells are capable of bouncing between its cytotoxic ($CD56^{dim}/CD16^{bright}$) and secretive ($CD56^{bright}/CD16^{dim}$) phenotype as well as in between its shades. *In vitro* exposition of NK cells to certain ligands can render them $CD56^{bright}/CD16^{bright}$ [29], suggesting that there is potential for *in vivo* phenotype shifting according to the molecular milieu. The fact that tissue's NK cells are predominantly of the secretive phenotype calls the attention for its immunomodulation importance and role. Hence, the desirable NK cell effect is also secretion of several cytokines and chemokines: active molecules that modulate immune adaptive system effectors, with higher specificity and less harmful for the organism.

In a recent Phase 1 trial of double bright ($CD56^{brigth}/CD16^{bright}$) NK cell (DB-NK) for refractory or relapsed AML (R/R-AML), we were able to document the persistence of the infused DB-NK cells, however, in

most responding patients T cell recovery predominated; we also showed that NK cell predominant in vivo expansion didn't necessary correlated with leukemia response (submitted manuscript); these results could suggest that antileukemic NK cell cytotoxic and immunomodulation activity results in an adaptive immunity response. In this phase 1 trial including a rather ill population of patients, cryopreserved, DB-NK cells infusions up to 10^7 cells/kg per infusion, in a total of 6 infusions, was well tolerated and its emergent anti leukemia or anti-microbial effects were clinically manageable. No CSR, fever, or serious adverse events were related with infusion, and in spite of some very ill patients been included, none of them died or clinically deteriorated because of NK cell adoptive immunotherapy. We treated 13 patients of whom 5 had primary refractory disease and 9 had relapsed or were refractory to SCT. The median line of previous treatments was 5, and they all received DB-NK cells with active disease. Seventy eight percent of the patients got either into CR (50%) or CRi (only one patient) or had partial response. OS for responders and non-responders were 344 and 254 days, respectively. PFS was 132 days for all and 199 days for responders. We were able to show that DB-NK cell adoptive immunotherapy is not only feasible and safe, but also effective in such an advance AML group of patients, increasing OS in spite of disease burden or localization as we also documented CNS responses. [30]

According to the MRD studies, SCT associated GVL anti-AML effect alone, cannot overcome high tumor burden, suggesting that adoptive immunotherapy ("graft") versus leukemia effect, so to speak, is probable more effective in a situation of minimal measurable disease, or preferentially, minimal residual disease (MRD).

The combination of target drugs, with or without low dose chemotherapy and immunotherapy should be pursued for augmenting good quality survival and possibly, cure in AML.

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