Use of Immunotherapy in Acute Myeloid Leukemia

Sharma, A., Pearson, A.N.

Cancer is a disease of uncontrolled cell growth driven by activating tumorigenic mutations which can cause metastasis, developing into tumors and can destroy normal body cells (Williams 2012). The immune system is the body's defense system against foreign species, characterized by cells, organs, and molecules. The immune system should ideally be able to detect cancerous cell as non-self and immunogenic, but many times, cancer cells surpass the detection by downregulating Major Histocompatibility Complexes (MHC) class I to avoid T cell recognition and upregulating immune checkpoints to induce T cell exhaustion (Muenst 2016). Immunotherapy is a treatment that boosts the patient's immune system to generate a response to specifically eliminate the cancer cells. Immunotherapy is an attractive option because of its targeted approach of destroying the cancer cells specifically as compared to the non-specific chemotherapies. In this review, we describe the use of immunotherapy to treat Acute Myeloid Leukemia (AML), with a focus on monoclonal antibodies, immune checkpoint inhibitors, and dendritic cell vaccination.

Acute Myeloid Leukemia (AML) is a type of blood cancer when early forms of leukocytes undergo uncontrollable growth with multiple genetic causes and many treatment options. AML occurs when genetic mutations in the precursor myeloid cells cause them to grow uncontrollably, disrupting the process of white blood cell maturation. Myeloid precursor cells are developed from hematopoietic stem cells (HSC) in the bone marrow, and are premature white blood cells (Dean 2005). Majority of the AML cases carry somatic variants (not hereditary) with over 50% being large genomic rearrangements or structural variants (SVs) and copy number variants (CNVs) (Levy). These mutations turn on oncogenes or turn off tumor suppressor genes such as *IDH1, IDH2, TET2, DNMT3A, WT1, NPM1, CEBPA and TP53* (Panuzzo 2020). Common genetic mutations in adult AML such as FLT3-ITD, are internal tandem duplication variants, NPM1, are frameshift mutations due to a small insertion that is found in around 27% of AML cases. DNMT3A, TET2, and IDH-1 and IDH-2,6, 7 are found in more than 40% of AML cases. The CEBPA is a transcription factor and frame-shift & nonsense mutation that is found in 6% of AML cases. TP53 mutations are found in only 2– 8% of cases and are missense mutations. RUNX1, PML-RARA, BCR-ABL1 are found in around 10-15% of AML cases, and are fusion mutations. AML more commonly affects adults, around 65 years and above, but also affects children with different prevalent mutations such as KMT2A-rearranged, alterations in RAS, KIT and WT1 genes (Aung 2021). It is more evident in males than females, and is prominent in caucasians over other ethnicities. The life expectancy varies depending on the age of the patient with a 5-year survival of 3-8% in patients aged 60 years and older, compared with 5-year survival rates of up to 50% for younger patients that are younger than 18 years old. Older patients tend to face poor prognostic factors, and typically request less intensive treatments from their doctors to avoid treatment-related stress on their fragile bodies (Oran 2012). Current treatments are split between chemotherapy, targeted therapy, and transplants of healthy cells. Intensive chemotherapy, also known as induction therapy, for AML, utilizes drugs such as high doses of cytarabine combined with anthracycline. Targeted therapy targets specific genetic mutations, such as FLT3 and IDH1/2, and are confirmed to have better prognosis results. Maintenance therapy, a lower intensity treatment which follows treatments such as radiation and

chemotherapy, uses drugs such as Oral azacitidine (CC-486) and FLT3 inhibitors (e.g., sorafenib, midostaurin, gilteritinib) for avoiding relapse in older patients. Hematopoietic Stem Cell Transplantation (HSCT) is an effective treatment that utilizes healthy stem cells which will later mature into the three hematopoietic cells. Immunotherapy is a promising treatment, but is not commonly practiced because it is still in clinical trial stages (Jaramillo 2023).

Immunotherapy treatments for AML include use of monoclonal antibodies, dendritic cell adoptive cell transfer, and immune checkpoint inhibitors. CD47, a ligand present on many hematopoietic cells, sends protective anti-phagocytic signals to immune cells such as macrophages and some dendritic cells (DC). The AML leukemic stem cells (LSCs) present an overexpression of CD47 on the cell surface, and are able to surpass phagocytosis, making them an effective immune checkpoint for the tumor cells to grow. AML LSCs also downregulate MHC class I molecules which avoid detection by cytotoxic T-cells. In contrast, natural killer cells (NK) are able to detect low levels of MHC and are effective in killing AML LSCs (Khaldoyanidi 2021). Monoclonal antibody development for immunotherapy treatment of AML targets the LILRB4 protein, present in monocytic AML cases, which plays a role in suppressing the immune responses. When LILRB4 is blocked, it can increase the cytotoxicity of T cells and become a more suitable target for the immune system. Researchers of University of Texas Southwestern Medical Center (UTSW) conducted experiments *in vitro*, and *in vivo* with mice models, by blocking APOE, the protein responsible for activating LILRB4. CRISPR-Cas9 technologies and anti-LILRB4 monoclonal antibody blockades are used in order to manipulate the genetic code of LILRB4 activation in APOE, and to shut off the protein as a whole (Deng 2018). Another immunotherapy for AML is immune checkpoint inhibitors (ICI). AML cells present ICIs such as Programmed cell death protein 1 (PD-1) and cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), which stop the cytotoxic T cells from killing the cancer. To stop ICIs from suppressing T cell function, Anti-PD-1 antibodies and Anti-CTLA-4 antibodies are administered to the patient. DC vaccination is another immunotherapy for AML which is when DC from the patient's blood are extracted, and altered *in vitro*. DC are then loaded with AML apoptotic corpses or various antigens such as WT1 mRNA, which is mostly prominent in big amounts on AML cells. These newly modified DC are then injected back into the patient's bloodstream. Because the DC are antigen-presenting cells (APC) and are stimulated with a target, DC communicate with T cells, and transfer information that gives the T cells a specific target to kill (Lichtenegger 2017). The CD8+ T cells are targeted by monoclonal antibodies when they suppress the LILRB4 protein, and increase cytotoxicity in T cells. T cells are also utilized by the modified DC in DC adoptive cell transfer in order to kill AML cells. DC are targeted by immunotherapy in AML because DC are altered *in vitro* by being loaded with AML specific antigens. Though many clinical trials are relatively new, one study showed that the monoclonal antibody treatment for blocking LILRB4 was shown to have a 50% success rate in complete remission (CR) (3 out of the 6 patients), and 62% of the patients were alive the following year given that the smaller sample size (Zhao 2024). Since the sample size is relatively small, more clinical trials with greater sample sizes are recommended to confirm these numbers. After evaluating 13 different studies that used ICI methods for immunotherapy for AML, 42% of the patients saw a positive impact of the treatment with the cancer getting better in some way. There was also 33% CR and an average of 8.9 more months of survival (Gómez-Llobell 2022). For DC adoptive cell transfer, 57% of the patients reached CR and 83% of those who received the DC vaccine had their cancer completely go away with a 48.9% chance of surviving for 3 more years (Van Acker 2019). In DC adoptive cell

transfer, the Monocyte-Derived Dendritic Cells (moDCs), which are DC found in monocytes and transfer the newly loaded information on the DC to cytotoxic T cells, can sometimes not be able to reach or successfully activate the T cells in the lymph nodes. They are also very inefficient when it comes to multiplying CD4+ T cells, which coordinate the immune response, so this leads to insufficient preparation of the newly informed T cells (Palomares 2024). One of the most important limitations of ICI immunotherapy for AML is the lack of data and clinical trials conducted specifically for pediatric cases (18 years or younger). Though adult cases show promising results, children's cases tend to result in mixed success, mostly due to the fact that there is no significant research or the immunotherapy does not work as efficiently in pediatric cases. In addition, PD-1/PD-L1 and CTLA-4 inhibitors have shown side effects of lung and intestinal inflammation, skin irritation, and fatigue (Park 2017). While the research on monoclonal antibody treatment targeting LILRB4 is still in the early clinical and research stages, as of 2017, one monoclonal antibody drug conjugate, gemtuzumab-ozogamicin targeting CD33 has been approved by the FDA specifically for AML. In 2015, the FDA approved another monoclonal antibody immunotherapy known as Daratumumab (DARA), targeting CD38 specifically approved for relapse of AML in patients 60 years or older.

An ongoing clinical trial, led by Van de Loosdrecht, strives to measure the efficacy of the allogeneic dendritic cell vaccine, DCP-001 in quiet AML patients, specifically to prevent relapse. Two cohorts of patients, 10 in each, were given different amounts of doses. Currently, this clinical trial is in Phase II, and has 20 enrolled patients, all above the age of 18. Patient selection criteria included patients who had already been through some sort of cancer therapy, but minimal residual disease (MRD) was allowed. Additionally, Patients with Acute Promyelocytic Leukemia (APL), a variation of AML, or with a history of previous allogeneic bone marrow or solid organ transplantation, and any Malignancies in the past 5 years with the exception properly treated carcinoma in situ of the cervix, squamous carcinoma of the skin, or properly controlled limited basal cell skin cancer, were not permitted to participate. Using the dendritic cell immunotherapy, patients are given either two different types of cells or vaccination of DCP-001 and booster vaccinations of 10e6 cells. Van de Loosdrecht and his fellow researchers used donor cells, which were far more efficient to produce and administer to the patient. Additionally, these clinical trial tests show how much dosage will be most effective in preventing relapse, which is most important because it is one of the primary areas of knowledge scientists lack. This trial allows the scientists to safely assess if the treatment works in small testing subjects with quiet AML or MRD, and eventually could provide the potential of using DC immunotherapy in active AML cases. Because MRD is inevitable, researchers heavily rely on this DC immunotherapy to be effective by completely eliminating cancer cells. This study showed promising results on its safety in elderly patients and generating a beneficial immune response. For future direction, researchers in this clinical trial are working on combining different treatment types such as both, DC and ICI immunotherapy, as it could potentially be more effective for the patient (NCT03697707). Another clinical trial, led by medical Dr. Sauer. in Germany, aims to use transplants and gene editing to modify the healthy cells of donors by deleting the CD33-deleted CD34+ hematopoietic stem cells (HSC). Donor cells will be taken from family members, and after transplant into patients, the patients will be given increasing doses of the anti-CD33 antibody drug conjugate Gemtuzumab-Ozogamicin (GO). Currently, this trial is in Phase I with 12 patients that are over the age of 18, having only 29% of their blood containing cancer and expressing the CD33 antigen on the leukemic blasts. Patients cannot have already had

exposure to GO, Acute or chronic Graft versus Host disease (GvHD), hepatic veno-occlusive disease (VOD). They need to have strong organs that have not been dysfunctional prior to the clinical trial, especially the liver and heart. This therapy uses an immune checkpoint inhibitor and gene editing to delete the CD33-deleted CD34+ HSC. This clinical trial is promising because it uses both the targeting of the CD33 antigen on AML cells and boosting the immune response by administering healthy donor cells which can potentially completely eliminate any remnant post-treatment cancer cells (NCT05662904).

AML is a blood cancer in which genetic mutations occur in precursor myeloid cells, which in turn, causes defects in white cell maturation. Immunotherapy treatments for AML are currently in the clinical trial stages but allogeneic hematopoietic stem cell transplantation (HSCT) is the most successful immunotherapies as of now, Antibody-Drug Conjugate immunotherapies, Dendritic cell vaccination, and ICIs have shown to be successful in early stage clinical trials. Immunotherapy has been a very beneficial form of treatment, especially for patients above the age of 60, who are in the post-relapse stages. Currently, researchers in the field of immunotherapy for AML are working on developing these treatments in order to progress to the clinical trial stages and looking further into combinations of different immunotherapies to treat AML. There are some obstacles to face such as AML not having enough specific antigens to be targeted and the lack of attention for pediatric AML cases. In the future, immunotherapy seems to be a promising treatment for AML as it has already shown success in current studies, and it is a working preventative for relapsing, elderly patients (Aureli 2021).

References

Acharya, U. H., Halpern, A. B., Wu, Q., Voutsinas, J. M., Walter, R. B., Yun, S., ... & Estey, E. H. (2018). Impact of region of diagnosis, ethnicity, age, and gender on survival in acute myeloid leukemia (AML). Journal of drug assessment, 7(1), 51-53.

Aung, M. M. K., Mills, M. L., Bittencourt-Silvestre, J., & Keeshan, K. (2021). Insights into the molecular profiles of adult and paediatric acute myeloid leukaemia. Molecular oncology, 15(9), 2253–2272. https://doi.org/10.1002/1878-0261.12899

Aureli, A., Marziani, B., Sconocchia, T., Del Principe, M. I., Buzzatti, E., Pasqualone, G., Venditti, A., & Sconocchia, G. (2021). Immunotherapy as a Turning Point in the Treatment of Acute Myeloid Leukemia. Cancers, 13(24), 6246. https://doi.org/10.3390/cancers13246246

Dean, L., & Dean, L. (2005). Blood groups and red cell antigens (Vol. 2). Bethesda: NCBI.

Deng, M., Gui, X., Kim, J., Xie, L., Chen, W., Li, Z., He, L., Chen, Y., Chen, H., Luo, W., Lu, Z., Xie, J., Churchill, H., Xu, Y., Zhou, Z., Wu, G., Yu, C., John, S., Hirayasu, K., Nguyen, N., … Zhang, C. C. (2018). LILRB4 signalling in leukaemia cells mediates T cell suppression and tumour infiltration. Nature, 562(7728), 605–609. https://doi.org/10.1038/s41586-018-0615-z

Gómez-Llobell, M., Peleteiro Raíndo, A., Climent Medina, J., Gómez Centurión, I., & Mosquera Orgueira, A. (2022). Immune Checkpoint Inhibitors in Acute Myeloid Leukemia: A Meta-Analysis. Frontiers in oncology, 12, 882531. https://doi.org/10.3389/fonc.2022.882531

Jaramillo S, Schlenk RF. Update on current treatments for adult acute myeloid leukemia: to treat acute myeloid leukemia intensively or non-intensively? That is the question. Haematologica 2023;108(2):342-352; https://doi.org/10.3324/haematol.2022.280802.

Khaldoyanidi, S., Nagorsen, D., Stein, A., Ossenkoppele, G., & Subklewe, M. (2021). Immune Biology of Acute Myeloid Leukemia: Implications for Immunotherapy. Journal of clinical oncology : official journal of the American Society of Clinical Oncology, 39(5), 419–432. https://doi.org/10.1200/JCO.20.00475

Levy, B., Baughn, L. B., Akkari, Y., Chartrand, S., LaBarge, B., Claxton, D., ... & Broach, J. R. (2023). Optical genome mapping in acute myeloid leukemia: a multicenter evaluation. Blood advances, 7(7), 1297-1307.

Li, M., & Zhao, X. (2024). LILRB4 in acute myeloid leukemia: From prognostic biomarker to immunotherapeutic target. Chinese Medical Journal. https://doi.org/10.1097/cm9.0000000000003195

Lichtenegger, F. S., Krupka, C., Haubner, S., Köhnke, T., & Subklewe, M. (2017). Recent developments in immunotherapy of acute myeloid leukemia. Journal of hematology & oncology, 10, 1-20.

Muenst, S., Läubli, H., Soysal, S. D., Zippelius, A., Tzankov, A., & Hoeller, S. (2016). The immune system and cancer evasion strategies: therapeutic concepts. Journal of internal medicine, 279(6), 541-562.

Oran B, Weisdorf DJ. Survival for older patients with acute myeloid leukemia: a population-based study. Haematologica. 2012 Dec;97(12):1916-24. doi: 10.3324/haematol.2012.066100. Epub 2012 Jul 6. PMID: 22773600; PMCID: PMC3590098.

Palomares, F., Pina, A., Dakhaoui, H., Leiva-Castro, C., Munera-Rodriguez, A. M., Cejudo-Guillen, M., Granados, B., Alba, G., Santa-Maria, C., Sobrino, F., & Lopez-Enriquez, S. (2024). Dendritic Cells as a Therapeutic Strategy in Acute Myeloid Leukemia: Vaccines. Vaccines, 12(2), 165. https://doi.org/10.3390/vaccines12020165

Panuzzo, C., Signorino, E., Calabrese, C., Ali, M. S., Petiti, J., Bracco, E., & Cilloni, D. (2020). Landscape of Tumor Suppressor Mutations in Acute Myeloid Leukemia. Journal of clinical medicine, 9(3), 802. https://doi.org/10.3390/jcm9030802

Park, J. A., & Cheung, N. V. (2017). Limitations and opportunities for immune checkpoint inhibitors in pediatric malignancies. Cancer treatment reviews, 58, 22–33. https://doi.org/10.1016/j.ctrv.2017.05.006

Pelcovits, A., & Niroula, R. (2020). Acute myeloid leukemia: a review. Rhode Island medical journal, 103(3), 38-40.

Van Acker, H. H., Versteven, M., Lichtenegger, F. S., Roex, G., Campillo-Davo, D., Lion, E., Subklewe, M., Van Tendeloo, V. F., Berneman, Z. N., & Anguille, S. (2019). Dendritic Cell-Based Immunotherapy of Acute Myeloid Leukemia. Journal of clinical medicine, 8(5), 579. https://doi.org/10.3390/jcm8050579

Williams, G. H., & Stoeber, K. (2012). The cell cycle and cancer. The Journal of pathology, 226(2), 352-364.

Xiangyu Zhao, Meng Lv, Yu Wang, Yi-Yang Ding, Zhixiao Zhou, Jiasheng Wang, HongLi Zheng, Xueqiang Zhao, Xin Lin, Xiao Jun Huang; LILRB4 Synthetic T-Cell Receptor and Antigen Receptor-T (STAR-T) for Refractory/Relapsed Acute Myeloid Leukemia: First-in-Human Phase I Clinical Trial. Blood 2024; 144 (Supplement 1): 4831. doi: https://doi.org/10.1182/blood-2024-207297

Zhong, X., & Ma, H. (2022). Targeting CD38 for acute leukemia. Frontiers in Oncology, 12. https://doi.org/10.3389/fonc.2022.1007783

NCT03697707

NCT05662904