Supplement to

Blood Cell Therapy
The official journal of APBMT

ABSTRACTS

from
the 26th Annual Congress of APBMT

October 15 – 17, 2021
VIRTUAL

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Experience with FACT-JACIE accreditation at the Singapore General Hospital

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The FACT-JACIE standards aim to promote quality medical and laboratory practice in hematopoietic progenitor cell transplantation and therapies using hematopoietic derived cellular products. Accreditation of hematopoietic stem cell transplant centers helps to inform patients, insurance organizations and governments of the high standards that these centers are dedicated to. We outline the journey and experiences with FACT-JACIE accreditation at the Singapore General Hospital and suggest how accreditation is part of a journey of continuous improvement rather than just a destination.

Standardization and Accreditation of CD34+ stem cell measurements for HSCT

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The advances in hematopoietic stem cell transplant (HSCT) in the 1990's including harvest of autologous and allogeneic blood stem cells have rendered the hematopoietic cell culture-based assay (CFUs) unsuitable for clinical purpose. Rapid quantitative measure of total $CD34^+$ stem cells (SC) by flow cytometry has become the preferred clinical test for blood SC harvest and biomarker of hematopoietic engraftment. The Royal College of Pathologists of Australasia (RCPA) has established an external quality assurance (EQA) program for CD34+ cells in early 2000's and this EQA program is used by over 60 national and international laboratories. However, this assay does not provide information on the viability of the CD34+ cells that is especially relevant for cryopreserved SC products. The 2018 edition of the FACT-JACIE International Standards for Hematopoietic Cellular Therapy Product Collection, Processing and Administration requires "…an assay measuring viable CD34 (vCD34+) be performed for HPC products intended for restoration of hematopoiesis". Recently published multi-centre studies demonstrated some inconsistencies in reported vCD34+ cell quantification among HSCT facilities, and hence, an EQA utilizing cryopreserved SC samples is urgently required. The current pandemic has also accelerated the demand for such program to drive inter-facility harmonization and standardization. However, several major obstacles need to be overcome and possible solutions to these challenges will be discussed.

HSCT STANDARDS IN LOW MIDDLE INCOME COUNTRIES

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The JACIE and FACT accreditation systems stand out as examples of profession-driven initiatives to improve quality in transplantation , which have subsequently been incorporated by third parties, such as healthcare payers (health insurers, social security) and competent authorities (treatment authorization). However, these standards have been extremely difficult to incorporate and follow in the LMIC region.

Much literature indicating a better clinical outcome in teaching hospitals and centers of excellence has been available since the 1990s. There is enough evidence of a positive relationship between the implementation of a quality management system and outcome of HSCT's, which showed that patients' outcome was systematically better when the transplantation center was at a more advanced phase of JACIE accreditation, independent of year of transplantation and other risk factors.

Similar results showed that centers accredited by both FACT and Clinical Trial Network (CTN) demonstrated significantly better results for more complex HSCT such as HLA-mismatched transplants.

These data reinforce the concept that clinical improvement is driven by the implementation of a quality management system embedded in external accreditation standards, especially in the context of more complex procedures. This process also results in a wider standardization of procedures across different countries and geographic areas, therefore contributing to providing patients with similar treatment expectations even when accessing different health management systems.

Hence there is a need for a system of standards for HSCT in the LMIC regions which must be implemented to improve patient outcomes.

Update on lentiviral gene therapy for the β-hemoglobinopathies

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The β-hemoglobinopathies, transfusion-dependent β-thalassemia (TDT) and sickle cell disease (SCD), are the most prevalent monogenic disorders worldwide. In TDT, a variety of mutations result in decreased or absent expression of β-globin, causing α- and β-globin chain imbalance with subsequent ineffective erythropoiesis and hemolytic anemia. SCD is caused by a single amino acid substitution (E6V) in β-globin that results in polymerization of sickle hemoglobin (HbS) upon deoxygenation. Red blood cells (RBCs) lose flexibility causing vaso-occlusive events (VOEs), chronic hemolytic anemia, and ultimately multi-organ damage.

We have designed lentiviral vectors that express an engineered $\beta^{A\text{-}T87Q}$-globin in the erythroid lineage under the control of the human locus control region and β-globin promoter. $\beta^{A\text{-}T87Q}$-globin is a strong inhibitor of HbS polymerization while allowing for its quantitative readout by high performance liquid chromatography (HPLC) analysis distinctively from endogenously expressed or transfused β-like globins[1]. We have obtained the sustained correction of SCD[2] and β-thalassemia[3] in mouse models by these vectors, and the first treated human patient with TDT became transfusion-free for > 6 years in a proof-of-principle trial[4]. After further optimization, yielding LentiGlobin BB305 vector and gene therapy (GT) protocol[5], we have aimed at assessing safety and efficacy in large human studies.

A total of 98 (63 TDT and 35 SCD) patients, ages 7 to 38 years, have been treated by GT with one-time autologous transplantation of apheresis-harvested CD34+ cells after Plerixafor mobilization (together with G-CSF for TDT) and subsequent ex vivo transduction by LentiGlobin BB305 in three Phase 1/2 (HGB-204, -205 for TDT; and -206 Group C for SCD) and two Phase 3 (HGB-207 and -212 for TDT) open-label clinical trials conducted in the USA (11 sites), France (2 sites), Germany (2 sites), Italy, Greece, the UK, Australia, and Thailand, with up to 7.5 years of follow-up[6,7,8,9].

Among TDT patients who completed a two-year Phase 3 study, regardless of genoptype (β^0/β^0, β^E/β^0, β^+/β^+, β^0/β^+) or age, 86.2% (25/29) became transfusion-independent (TI), defined as weighted average blood hemoglobin (Hb) ≥ 9 g/dL without packed RBC transfusions for ≥ 12 months, and they remain TI up to their last study visit. In TI patients, weighted average blood Hb was 11.8 (9.35 – 13.7) g/dL, most of which comprised of $\beta^{A\text{-}T87Q}$-globin. After TI, a decrease in liver iron concentrations approaching normal values was seen in most TDT patients, and cardiac iron levels remained within the normal range. Serum ferritin and soluble transferrin receptor levels demonstrated improvement of ineffective erythropoiesis. No serious (SAEs) or other adverse events (AEs) related to the drug product were observed. No malignancy or clonal expansion were detected.

Most SCD patients were β^S/β^S. In the protocol-optimized HGB-206's Group C, there was complete resolution and prevention of VOEs in all (n = 19) patients having at least 6 month follow-up post GT in the absence of any supportive treatment (no exchange transfusion or hydroxyurea). Major improvement in pain intensity was observed irrespective of baseline values relative to population norm. Accordingly, median $Hb^{A\text{-}T87Q}$-globin represented ≥ 40% of all Hb species after at least 6 month follow-up post GT. $\beta^{A\text{-}T87Q}$-globin was largely pan-cellular across circulating RBCs, and high levels of endogenous γ-globin (F cells) were not necessary for a therapeutic effect. Hemolysis and erythropoiesis markers (reticulocyte count, serum

lacatate dehydrogenase, serum indirect bilirubin, nucleated RBCs, serum transferin receptor) approached normal values after LentiGlobin BB305 treatment. No SAEs or AEs related to the drug product were observed, although two patients in HGB206 were diagnosed with acute myeloid leukemia (AML). The most recent diagnosis led to the temporary trial suspension of HGB-206 and HGB-210. The cause of this AML was subsequently found to be unlikely related to vector integration after in-depth genetic analyses, and the trials have resumed. These SAEs also have to be considered in light of recent reports that SCD patients have an increased risk of developing hematologic malignancies compared to the general population.

Altogether, there results have led the US FDA to grant LentiGlobin BB305 “Breakthrough Therapy Designation (BTD)” for TDT and “Regenerative Medicine Advanced Therapy Designation (RMATD)” for SCD. In Europe, conditional Market Authorization was granted in May 2019 to LentiGlobin BB305 for non-β0 TDT under the product name betibeglogene autotemcel (Zynteglo).

There is, however, further room for vector improvement. Accordingly, we have recently reported the design of a BB305 derivative that also expresses an intronic shRNA directed against α2-globin, in an effort to correct TDT phenotypes with greater potency and hence lower VCN by better correcting the α- and β-globin chain imbalance at single vector copy[10].

In sum, clinical trial outcomes of near 100 patients with a broad variety of ages and genotypes, treated by GT with Lentiglobin BB305 at multiple international sites and observed for up to 7.5 years, support the long-term safety and durability of effect for lentiviral-based additive GT for the β-hemoglobinopathies. If confirmed by subsequent trials and post-market studies, lentiviral additive GT may become standard of care for SCD and TDT.

References

1. Takekoshi, K.J., Oh, Y.H., Westerman, K.W., London, I.M. & Leboulch, P. Retroviral transfer of a human beta-globin/delta-globin hybrid gene linked to beta locus control region hypersensitive site 2 aimed at the gene therapy of sickle cell disease. Proc Natl Acad Sci U S A 92, 3014-3018. (1995).
2. Pawliuk, R., et al. Correction of sickle cell disease in transgenic mouse models by gene therapy. Science 294, 2368-2371. (2001).
3. Imren, S., et al. Permanent and panerythroid correction of murine beta thalassemia by multiple lentiviral integration in hematopoietic stem cells. Proc Natl Acad Sci U S A 99, 14380-14385 (2002).
4. Cavazzana-Calvo, M., et al. Transfusion independence and HMGA2 activation after gene therapy of human beta-thalassaemia. Nature 467, 318-322 (2010).
5. Negre, O., et al. Preclinical Evaluation of Efficacy and Safety of an Improved Lentiviral Vector for the Treatment of beta-Thalassemia and Sickle Cell Disease. Curr Gene Ther 15, 64-81 (2015).
6. Thompson, A.A., et al. Gene Therapy in Patients with Transfusion-Dependent beta-Thalassemia. N Engl J Med 378, 1479-1493 (2018).
7. Ribeil, J.A., et al. Gene Therapy in a Patient with Sickle Cell Disease. N Engl J Med 376, 848-855 (2017).
8. Thompson, A.A., et al. Resolution of Serious Vaso-Occlusive Pain Crises and Reduction in Patient-Reported Pain Intensity: Results from the Ongoing Phase ½ HGB-206 Group C Study of LentiGlobin for Sickle Cell Disease (bb1111) Gene Therapy. Blood, 16–17 (2020).
9. Thompson, A.A., et al. Favorable Outcomes in Pediatric Patients in the Phase 3 Hgb-207 (Northstar-2) and Hgb-212 (Northstar-3) Studies of Betibeglogene Autotemcel Gene Therapy for the Treatment of Transfusion-Dependent β-Thalassemia. Blood 136, 52–54. (2020).
10. Nualkaew, T., et al. Coordinated beta-globin expression and alpha2-globin reduction in a multiplex lentiviral gene therapy vector for beta-thalassemia. Mol Ther (2021).

CRISPR-based gene therapy

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The recent advance in clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated (Cas) 9 technology has revolutionized the genome editing with great potential for therapeutic applications. This traditional CRISPR/Cas9 technology consists of two components: a Cas9 endonuclease and a single guide RNA (sgRNA) that guides the Cas9 to cleave and introduce a double-strand break at the target sequence. The DSB is then repaired by two competing DNA repair pathways in the cell: non-homologous end joining (NHEJ) leading to sequence/gene disruption by small insertions/deletions (indels) and homology-directed repair (HDR) leading to gene correction when a donor DNA template is provided. To date, CRISPR/Cas9 technology has been investigated in clinical trials for treating a number of diseases such as cancers, β-hemoglobinopathies, inherited retinal disorder. However, one of the major concerns for CRISPR/Cas9-based therapeutics is that DSBs induced by CRISPR/Cas9 may cause activation of p53 DNA damage response and unwanted DNA alterations, including genomic deletions, chromosomal translocations, which can potentially lead to adverse effects. Additionally, the efficiency of HDR-mediated gene correction is limited by the competition with NHEJ, which can cause indels at the target sequence, especially in non-dividing cells. Therefore, several variants of CRISPR/Cas9 technologies have been developed to overcome these limitations.

There are two main Cas9 variants that cannot induce DSBs while retaining the ability to recognize specific target sequences based on the guiding of sgRNAs. The first variant is dead Cas9 (dCas9), which has double point mutations: D10A and H840A in RuvC and HNH nuclease domains of Cas9, respectively, leading to loss of its nuclease activity. The dCas9 can be fused to transcriptional activators, transcriptional repressors, or epigenetic modifiers to mediate gene repression (CRISPR interference or CRISPRi), gene activation (CRISPRa), or epigenetic modification, respectively. The second variants are Cas9 nickases, which have either D10A or H840A mutations, generating a nick in a single DNA strand rather than the DSB. The Cas9 nickases can be fused to nucleobase deaminase enzymes, which can convert a cytosine to a thymine (cytosine base editor: CBE), or an adenosine to a guanosine (adenosine base editor: ABE) without introducing DSBs. These base editors can be therapeutically applied to correct point mutations, introduce a premature stop codon, or modify splicing pathways. Moreover, Cas9 nickases can be fused to a reverse transcriptase (prime editor), which can synthesize a new DNA strand from RNA template in a modified sgRNA called a prime editing guide RNA (pegRNA). The new DNA is then incorporated into the nicked DNA. While currently less efficient than base editor, the prime editor is more precise and flexible for correction of specific mutations[1].

One of the most advanced therapeutic applications of the CRISPR technology is for the treatment of β-hemoglobinopathies, including sickle cell diseases (SCD) and β-thalassemia. Here we summarize the latest studies with an aim to give a brief update on therapeutic promise of CRISPR technology for β-hemoglobinopathies. The CRISPR technology for treatments of β-hemoglobinopathies can be divided into correction of β-globin (*HBB*) mutations to restore adult hemoglobin (HbA, $\alpha_2\beta_2$) or functional hemoglobin, addition of a normal *HBB* transgene, and induction of fetal hemoglobin (HbF, $\alpha_2\gamma_2$). To correct *HBB*

mutations, the CRISPR/Cas9 has been employed to correct several common *HBB* mutations such as SCD mutation and CD41/42 (-CTTT) in $CD34^+$ hematopoietic stem/progenitor cells (HSPCs) through HDR pathway using a sgRNA and Cas9 ribonucleoprotein (RNP) complex along with a single-strand oligodeoxynucleotide[2]. Since the efficiency of HDR-based editing is low (10-20%), strategies to enhance HDR rates in HSPCs and/or to select corrected cells would be required to improve the correction efficiency. Moreover, the adenosine base editor has been shown to efficiently convert the mutant SCD codon (GTG, valine) to alanine (GCG) to generate Hb G-Makassar, a naturally occurring, non-pathogenic hemoglobin variant, in $CD34^+$ HSPCs with 80% conversion without any p53 activation or large deletions[3]. Interestingly, this adenosine base editor system led to phenotypic improvements of humanized SCD mice. Additionally, the CRISPR/Cas9 has been employed to introduce a normal *HBB* complementary DNA (cDNA) delivered by an adeno-associated virus serotype 6 (AAV6) into the endogenous locus by HDR-mediated insertion, which can be applied as a more universal therapeutic strategy for β-hemoglobinopathies[4].

Clinical studies have shown that co-inheritance of hereditary persistence of fetal hemoglobin (HPFH), a naturally occurring condition with elevation of HbF (the predominant hemoglobin in the fetus) throughout adult life, can ameliorate the clinical severity of β-hemoglobinopathies. The CRISPR/Cas9 has been employed to induce therapeutic levels of HbF expression through NHEJ pathway to mimic nondeletional or deletional HPFH genotypes in $CD34^+$ HSPCs[5-7]. Recently, phase 1/2 clinical trials of CRISPR/Cas9 to induce HbF expression have been investigated for the treatment of transfusion-dependent β-thalassemia (NCT03655678) and SCD (NCT03745287)[8]. This was achieved by using the Cas9 and sgRNA RNP complex to disrupt an erythroid specific enhancer of BCL11A, a major γ-globin repressor[9]. Autologous transplantation of edited $CD34^+$ HSPCs (CTX001) into patients led to sustained and high levels in HbF expression, and the elimination of vaso-occlusive episodes and need for transfusions at more than a year after the infusion[8]. However, a long-term follow-up would be needed to monitor the long-term safety profile of CTX001. Moreover, the modified cytosine base editor was used to deactivate the BCL11A erythroid-specific enhancer, resulting in therapeutic levels of HbF induction in $CD34^+$ HSPCs from β-thalassemia and SCD patients[10].

In summary, advances in the development of traditional and next-generation CRISPR/Cas9 technologies to facilitate genome modification could lead to therapeutic applications, which may provide curative treatments for a range of diseases.

References

1. Zeballos CM, Gaj T. Next-Generation CRISPR Technologies and Their Applications in Gene and Cell Therapy. Trends Biotechnol. 2021 Jul;39(7):692-705.
2. DeWitt MA, Magis W, Bray NL, Wang T, Berman JR, Urbinati F, et al. Selection-free genome editing of the sickle mutation in human adult hematopoietic stem/progenitor cells. Sci Transl Med. 2016 Oct 12;8(360):360ra134.
3. Newby GA, Yen JS, Woodard KJ, Mayuranathan T, Lazzarotto CR, Li Y, et al. Base editing of haematopoietic stem cells rescues sickle cell disease in mice. Nature. 2021 Jul;595(7866):295-302.
4. Dever DP, Bak RO, Reinisch A, Camarena J, Washington G, Nicolas CE, et al. CRISPR/Cas9 beta-globin gene targeting in human haematopoietic stem cells. Nature. 2016 Nov 17;539(7629):384-389.
5. Metais JY, Doerfler PA, Mayuranathan T, Bauer DE, Fowler SC, Hsieh MM, et al. Genome editing of HBG1 and HBG2 to induce fetal hemoglobin. Blood Adv. 2019 Nov 12;3(21):3379-3392.

6. Weber L, Frati G, Felix T, Hardouin G, Casini A, Wollenschlaeger C, et al. Editing a gamma-globin repressor binding site restores fetal hemoglobin synthesis and corrects the sickle cell disease phenotype. Sci Adv. 2020 Feb;6(7).
7. Antoniani C, Meneghini V, Lattanzi A, Felix T, Romano O, Magrin E, et al. Induction of fetal hemoglobin synthesis by CRISPR/Cas9-mediated editing of the human beta-globin locus. Blood. 2018 Apr 26;131(17):1960-1973.
8. Frangoul H, Ho TW, Corbacioglu S. CRISPR-Cas9 Gene Editing for Sickle Cell Disease and beta-Thalassemia. Reply. N Engl J Med. 2021 Jun 10;384(23):e91.
9. Wu Y, Zeng J, Roscoe BP, Liu P, Yao Q, Lazzarotto CR, et al. Highly efficient therapeutic gene editing of human hematopoietic stem cells. Nat Med. 2019 May;25(5):776-783.
10. Zeng J, Wu Y, Ren C, Bonanno J, Shen AH, Shea D, et al. Therapeutic base editing of human hematopoietic stem cells. Nat Med. 2020 Apr;26(4):535-541.

Regeneration of cytotoxic T lymphocytes from iPS cells: Development of "off-the-shelf T cells" for cell therapy targeting cancer and viral infection

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In currently ongoing adoptive T cell therapies, T cells collected from a patient are transduced with chimeric antigen receptor (CAR) or T cell receptor (TCR) genes and given back to the patient after further ex-vivo activation and expansion. While such strategies using autologous T cells have been shown to be effective for some types of cancer[1, 2)], some issues remain to be solved: these methods are i) time-consuming and ii) costly, and iii) difficult to guarantee the quality because the products depend on patient-derived T cells.

To solve the problems mentioned above, we have thought of a method in which T cells can be mass-produced by using the iPS cell technology. Our initial concept is the following; when iPSCs are generated from T cells (T-iPSCs), the genomic structure of rearranged T cell receptor (TCR) genes should be passed on to the iPSCs, and T cells regenerated from these iPSCs should express the same TCR as the original T cells. In line with this concept, at first we produced iPSCs from human cytotoxic T lymphocytes (CTLs) specific for melanoma antigen MART1[3)]. We then regenerated CD8 T cells from such MART1-T-iPSCs and found that almost all regenerated T cells expressed TCR specific for MART1 antigen.

While we reported our initial success in 2013 as mentioned above, later on we came to notice that the regenerated T cells expressed CD8aa homodimer. It is known that CD8ab can bind to the HLA molecule and thus efficiently strengthens the TCR signal, but CD8 aa does not. Therefore, we improved our culture procedure and succeeded in inducing CD8ab T cells that were thought to satisfy criteria to call them CTLs[4)]. Hence, hereafter we use the term CTLs for the regenerated CD8 T cells. Whereas in our original procedure, whole cells were stimulated by anti-CD3 mAb when we saw emergence of CD4/CD8 double-positive (DP) cells in the culture, in our newly developed method, DP cells were firstly separated from DN cells and then stimulated by anti-CD3 mAb, resulting in efficient production of CD8ab CTLs.

In order to assess the feasibility of this strategy, we investigated the frequency of usable T-iPSC clones in terms of their T cell generating capability and TCR affinity. Among eight clones of T-iPSCs established from a healthy volunteer, five clones were considered to be usable[5)]. Functional avidities measured by cytotoxic activity of the regenerated CTLs were almost equivalent among three selected clones representing high, medium and low TCR affinity. These findings support the feasibility of this T-iPSC strategy.

We have thus far described the T-iPSC method in which the material iPSCs are produced from T cells. As a next step, we thought of an alternative approach: to transduce iPSCs with an exogenous TCR gene (TCR-iPSC method). With the TCR-iPSC method, it would be much easier to establish high quality iPSC clones bearing a rearranged TCR gene, since it becomes possible to use iPSCs and TCR genes of guaranteed quality. To examine whether this idea works in practice, we cloned WT1-specific TCR genes from CTLs regenerated from WT1-T-iPSCs, and transferred them into iPSCs that had been originally derived from monocytes of an HLA-haplotype homozygous healthy volunteer (WT1-TCR-iPSCs)[6)]. Using these WT1-TCR-iPSCs, we succeeded in regenerating CD8ab CTLs, which exhibited antigen-specific

cytotoxic activity comparable to CD8ab CTLs regenerated from T-iPSCs, demonstrating that TCR-iPSC method works as well as T-iPSC method does.

We then extended this TCR-iPSC strategy to solid tumors. To this end, we developed an allogeneic approach by transducing HLA-haplotype homozygous iPSCs that had been provided by Center for iPSC Research and Application, Kyoto University[7], with WT1-specific TCR gene that had been tested clinically[8]. The regenerated CTLs antigen-specifically suppressed tumor growth in a patient-derived xenograft model of renal cell carcinoma, demonstrating the feasibility of our strategy against solid tumors[9].

At present, we are working to set up a clinical trial targeting WT1-antigen in acute myeloid leukemia (AML) patients in collaboration with the Department of Hematology and Oncology and the Center for Research and Application of Cellular Therapy in Kyoto University Hospital. When aged AML patients, who may have no option of stem cell transplantation, undergo relapse after initial chemotherapy, no effective therapy is available so far. Such patients could be appropriate candidates for our trial.

As above, we have demonstrated that the TCR-iPSC method works very well[6, 9] and actually plan to use this method in clinical trial. Even so, we decided to develop a next generation method, because this TCR-iPSC method still has some points that can be further addressed: i) risk of damaging genome by lentiviral transduction, and ii) some difficulty in controlling expression level of TCR. It is therefore preferable that an exogenous TCR gene is integrated into TCR gene locus so that it could be expressed under the control of endogenous promoter/enhancer while causing no genome damage. It would be also nice if we could insert a TCR gene just like “a cassette tape”. To this end, we have developed a novel method named “TCR cassette method”, in which we firstly knocked-in a “cassette deck” structure containing Vb promotor into TCR gene locus upstream of enhancer of TCRb gene in non-T derived iPSCs. We then inserted NY-ESO1-TCR as a cassette tape into the cassette deck. The resulted iPSCs gave rise to potent CTLs having NY-ESO1-specific cytotoxic activity, confirming that this new method is applicable in producing CTLs for cell therapy against cancer.

Since 2020, the whole world has been hit by the pandemic of COVID-19. To fight against this disease, we have decided to apply our method to the disease, and have started to develop an off-the-shelf T cell medicine for COVID-19. Thus, we have launched a project to clone TCRs specific for SARS-Cov-2 in Fujita Health University, which has conducted clinical trial of Avigan, an anti-virus drug for COVID-19, and thus has been a dominant organization in terms of development of new strategy against COVID-19. Provided a corona-specific TCR is restricted to HLA-A2402, the most frequent HLA-A allele in Japanese population, such TCR is expected to be used for 60% of patients in Japan. We propose that this strategy is also applicable to other viral infections, such as SARS, MERS, etc.

References

1. Grupp SA, Kalos M, Barrett D, et al. Chimeric antigen receptor-modified T cells for acute lymphoid leukemia. N Engl J Med 2013 ; 368 : 1509.
2. Rosenberg SA. Cell transfer immunotherapy for metastatic solid cancer--what clinicians need to know. Nat Rev Clin Oncol 2011 ; 8 : 577.
3. Vizcardo R, Masuda K, Yamada D, et al. Regeneration of human tumor antigen-specific T cells from iPSCs derived from mature $CD8^+$ T cells. Cell Stem Cell 2013;12:31–6.
4. Maeda T, Nagano S, Ichise H, et al. Regeneration of CD8αβ T cells from T-cell derived iPSC imparts potent tumor antigen-specific cytotoxicity. Cancer Res 2016;76:6839–50.

5. Nagano S, Maeda T, Ichise H, Kashima S, Ohtaka M, Nakanishi M, Kitawaki T, Kadowaki N, Takaori-Kondo A, Masuda K, Kawamoto H. High Frequency Production of T Cell-Derived iPSC Clones Capable of Generating Potent Cytotoxic T Cells. Mol Ther Methods Clin Dev. 2019 ; 16:126-135.
6. Maeda T, Nagano S, Kashima S, et al. Regeneration of Tumor-Antigen-Specific Cytotoxic T Lymphocytes from iPSCs Transduced with Exogenous TCR Genes. Mol Ther Methods Clin Dev 2020 ; 19 : 250.
7. Okita K, Matsumura Y, Sato Y et al. A more efficient method to generate integration-free human iPS cells. Nat Methods. 2011; 8: 409-12.
8. Tawara I, Kageyama S, Miyahara Y, et al. Safety and persistence of WT1-specific T-cell receptor gene−transduced lymphocytes in patients with AML and MDS. Blood 2017;130:1985–94.
9. Kashima S, Maeda T, Masuda K et al. Cytotoxic T Lymphocytes Regenerated from iPS Cells Have Therapeutic Efficacy in a Patient-Derived Xenograft Solid Tumor Model. iScience. 2020; 23: 100998.

Biomarkers and GVHD

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Acute graft-versus-host disease (GVHD) remains a life-threatening morbidity of allogeneic hematopoietic cell transplant (HCT), affects three organ systems (skin, liver, gastrointestinal (GI) tract), and requires systemic steroid treatment in approximately 45% of all transplant recipients [1]. Even though recent advances in GVHD prevention, such as post-transplant cyclophosphamide, have reduced its maximal severity[2,3], non-relapse mortality (NRM), driven by GVHD of the GI tract, remains unacceptably high at 15%[4]. The clinical severity at GVHD onset predicts outcomes too poorly to guide treatment and thus everyone is treated with high dose systemic steroids, under treating some and over treating others.

Over the past few years, our group validated a biomarker-based risk stratification system that is more accurate than clinical symptoms at classifying GVHD severity[5]. The Mount Sinai Acute GVHD International Consortium (MAGIC) algorithm probabilities (MAPs) define the Ann Arbor risk scores (1, 2 or 3) that identify patients at high risk for treatment failure as well as patients who respond well to steroids and would benefit from reductions in treatment intensity. Lethal GVHD results from extensive damage to the GI crypt, which is the primary source of both ST2 (the soluble IL33 receptor) and REG3α (a Paneth cell protein) that are released into the serum[5-7]. Preclinical models have demonstrated that Reg3α enters the systemic circulation when Paneth cells are destroyed; intestinal stromal and endothelial cells as well as effector lymphocytes are the main source of ST2[6-8]. Thus, the MAP can be considered a “liquid biopsy” of GI crypts throughout the intestine and inversely correlates with ISC numbers, which in turn reflects the capacity for crypt regeneration and tissue repair. The presence of GI crypt damage portends a worse outcome, even when GI symptoms are minimal or absent, while the absence of GI crypt damage predicts response to steroid treatment, even when GI symptoms are present [5,9,10].

In addition to its utility at the onset of GVHD, the MAP also predicts outcomes during treatment. In one study, MAPs obtained after one week of steroid treatment in three independent cohorts stratified patients with steroid-resistant GVHD into two groups with distinctly different outcomes. Patients with steroid-resistant GVHD and a high MAP had exceedingly poor outcomes compared to patients whose GVHD had not improved after one week of treatment but had a low MAP (12 month NRM 75% vs 14%, $p<0.001$)[11]. Low MAPs presumably reflect a biological response to treatment that could be detected in patients who proved to be “slow clinical responders”. A second study that examined the MAP as a response biomarker supports this hypothesis. The clinical response after 4 weeks of treatment is commonly used as the primary endpoint for primary treatment clinical trials[12]. We compared clinical response to MAPs as predictors of NRM and survival after 1, 2 and 4 weeks of treatment in 615 patients divided into test (n=248) and validation cohorts (n=367)[13]. MAPs more accurately predicted 6-month NRM than clinical response at all time points tested; indeed the MAP after 1 week of treatment was a better predictor of outcome than clinical response at 4 weeks of treatment. Furthermore, change in MAPs correlated with outcomes. Patients whose MAPs increased during treatment were more likely to die than patients whose MAPs stayed the same or declined.

Provided that biomarker risk scores can be generated in real time, they can be used as inclusion criteria for clinical trials that tackle the two most pressing problems in GVHD, under and over-treatment. The MAGIC consortium has completed accrual to three clinical trials that determined GVHD treatment intensity based on high or low risk biomarker scores. The first trial, which recently completed accrual, tested whether high MAPs at day 7 or 14 post-allogeneic HCT in asymptomatic patients could be used to preempt the development of severe GVHD.

Biomarkers derived from GI tissue damage are not the only biomarkers associated with GVHD outcomes. Other studies have correlated biomarkers associated with activation of the immune system and endothelial damage with GVHD outcomes, with the latter showing particular promise[14-16]. However, these other biomarkers still require comparison to the MAP and are not yet widely available for clinical use.

References

1. Majhail NS, Chitphakdithai P, Logan B, et al. Significant improvement in survival after unrelated donor hematopoietic cell transplantation in the recent era. Biol Blood Marrow Transplant 2015;21:142-50.
2. Bashey ZA, Zhang X, Brown S, et al. Comparison of outcomes following transplantation with T-replete HLA-haploidentical donors using post-transplant cyclophosphamide to matched related and unrelated donors for patients with AML and MDS aged 60 years or older. Bone Marrow Transplant 2018;53:756-63.
3. McCurdy SR, Kanakry CG, Tsai HL, et al. Grade II acute graft-versus-host disease and higher nucleated cell graft dose improve progression-free survival after HLA-haploidentical transplant with post-transplant cyclophosphamide. Biol Blood Marrow Transplant 2018;24:343-52.
4. Aziz MD, Shah J, Kapoor U, et al. Disease risk and GVHD biomarkers can stratify patients for risk of relapse and nonrelapse mortality post hematopoietic cell transplant. Leukemia 2020;34:1898-906.
5. Hartwell MJ, Ozbek U, Holler E, et al. An early-biomarker algorithm predicts lethal graft-versus-host disease and survival. JCI Insight 2017;2:e89798.
6. Ferrara JL, Harris AC, Greenson JK, et al. Regenerating islet-derived 3-alpha is a biomarker of gastrointestinal graft-versus-host disease. Blood 2011;118:6702-8.
7. Zhang J, Ramadan AM, Griesenauer B, et al. ST2 blockade reduces sST2-producing T cells while maintaining protective mST2-expressing T cells during graft-versus-host disease. Sci Transl Med 2015;7:308ra160.
8. Zhao D, Kim YH, Jeong S, et al. Survival signal REG3alpha prevents crypt apoptosis to control acute gastrointestinal graft-versus-host disease. J Clin Invest 2018;128:4970-9.
9. Levine JE, Braun TM, Harris AC, et al. A prognostic score for acute graft-versus-host disease based on biomarkers: a multicentre study. The Lancet Haematology 2015;2:e21-9.
10. Levine JE, Huber E, Hammer ST, et al. Low Paneth cell numbers at onset of gastrointestinal graft-versus-host disease identify patients at high risk for nonrelapse mortality. Blood 2013;122:1505-9.
11. Major-Monfried H, Renteria AS, Pawarode A, et al. MAGIC biomarkers predict long-term outcomes for steroid-resistant acute GVHD. Blood 2018;131:2846-55.
12. MacMillan ML, DeFor TE, Weisdorf DJ. The best endpoint for acute GVHD treatment trials. Blood 2010;115:5412-7.
13. Srinagesh HK, Ozbek U, Kapoor U, et al. The MAGIC algorithm probability is a validated response biomarker of treatment of acute graft-versus-host disease. Blood Adv 2019;3:4034-42.
14. Holtan SG, DeFor TE, Panoskaltsis-Mortari A, et al. Amphiregulin modifies the Minnesota Acute Graft-versus-Host Disease Risk Score: results from BMT CTN 0302/0802. Blood Adv 2018;2:1882-8.

15. Abu Zaid M, Wu J, Wu C, et al. Plasma biomarkers of risk for death in a multicenter phase 3 trial with uniform transplant characteristics post-allogeneic HCT. Blood 2017;129:162-70.
16. McDonald GB, Tabellini L, Storer BE, Lawler RL, Martin PJ, Hansen JA. Plasma biomarkers of acute GVHD and nonrelapse mortality: predictive value of measurements before GVHD onset and treatment. Blood 2015;126:113-20.

Novel Approach in GVHD prophylaxis

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The pathophysiology of GVHD beyond donor effector T cells is now better understood. Recent data indicate that tissue stem cells are targeted in GVHD, resulting in dysregulation of tissue homeostasis and associated microbial ecology. Particularly, damage to the intestine plays a central role in propagating a proinflammatory cytokine milieu and amplifying systemic GVHD; intestinal GVHD is the major cause of non-relapse mortality after allogeneic HCT. Intestinal secretary cells such as Paneth cells and Goblet cells shape intestinal microbiota and protect host from pathogen invasion. Thus, the sensitivity of target tissues to GVHD may be modulated by tissue-intrinsic resilience and homeostasis. Integration of both immune tolerance and tissue tolerance could optimize GVHD prophylaxis. R-spondin and IL-22 are growth factors for intestinal stem cells (ISCs) and prevent experimental GVHD. IL-25, a growth factor of goblet cells, protects goblet cells and prevents bacterial invasion in an anti-microbial molecules Lypd8 dependent manner. Such strategies to promote tissue tolerance promise to open a new avenue to improve transplant outcomes.

References

1. S. Takashima, et al: The Wnt agonist R-spondin1 regulates systemic graft-versus-host disease by protecting intestinal stem cells. J Exp Med, 208(2), 285-94 (2011).
2. Y. Eriguchi, et al: Graft-versus-host disease disrupts intestinal microbial ecology by inhibiting Paneth cell production of alpha-defensins. Blood, 120(1), 223-31 (2012).
3. E. Hayase, et al: R-Spondin1 expands Paneth cells and prevents dysbiosis induced by graft-versus-host disease. J Exp Med, 214(12), 3507-3518 (2017).
4. C. K. Stein-Thoeringer, et al: Lactose drives Enterococcus expansion to promote graft-versus-host disease. Science, 366(6469), 1143-1149 (2019).
5. J. U. Peled, et al: Microbiota as Predictor of Mortality in Allogeneic Hematopoietic-Cell Transplantation. N Engl J Med, 382(9), 822-834 (2020).
6. T. Ara, et al: Intestinal goblet cells protect against GVHD after allogeneic stem cell transplantation via Lypd8. Sci Transl Med, 12(550) (2020).

Biology of CAR T Toxicities

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Chimeric antigen receptor (CAR) T-cell therapy is a form of engineered adoptive cellular therapy that has shown remarkable efficacy in the treatment of cancer, especially hematologic malignancies. The efficacy of this therapy depends on the CAR T-cells' recognition of the target antigen, followed by CAR T-cell activation, expansion and immune-killing of the cancer cells, as well as sufficient persistence to provide immune surveillance for tumor control. This T-cell immune response in turn sets the stage for the toxicities that frequently accompany CAR T-cell therapies, namely cytokine release syndrome (CRS) and immune effector cell-associated neurotoxicity syndrome (ICANS).

CRS is a type of systemic inflammatory response syndrome and is characterised by fever with or without hypotension, hypoxia or other organ dysfunction. The C-reactive protein (CRP) and serum ferritin are common laboratory markers reflective of systemic inflammation and are elevated during CRS. Preclinical and clinical research have shown elevated levels of various cytokines in CRS. Activated CAR T-cells produce interferon (IFN) gamma, tumor necrosis factor (TNF) alpha and granulocyte-macrophage colony stimulating factor (GM-CSF). These activate and stimulate monocytes and macrophages to produce interleukin-6 (IL6), IL1 and IL10, which drive the manifestations of CRS. Correspondingly, treatment of CRS using the IL6 receptor antagonist tocilizumab tends to produce rapid and efficacious response.

Compared to CRS, the biology of ICANS has been less clearly understood. Elevated levels of cytokines (IL1, IL6, GM-CSF) and myeloid cells in the systemic circulation and the cerebrospinal fluid (CSF) have been correlated with the development of ICANS. However, ICANS does not respond as well to tocilizumab treatment, suggesting that IL6 is not a dominant player in the pathophysiology of ICANS. Endothelial dysfunction and blood brain barrier dysfunction have been identified as potential mechanisms contributing to ICANS.

Other CAR T toxicities such as cytopenias and on-target off-tumor effects would also be discussed.

Toxicities of chimeric antigen receptor T cell therapy-manifestations, assessments and management

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The toxicities of CAR-T cell therapy include cytokine release syndrome (CRS), Immune Effector Cell-Associated Neurotoxicity Syndrome (ICANS), cytopenia, B cell aplasia, Haemophagocytic lymphohistiocytosis / macrophage-activation syndrome (HLH/MAS) and so on. Specifically, CRS and ICANS are the most two common side effects of CAR-T cell therapy, relevant to the overactive immunocytes and release of cytokines in cascades, resulting in the systematic or neurological symptoms. Cytopenia could often be seen in patients while might greatly influence the prognosis of patients as severe infection and bleeding should be highly paid attention to. B cell aplasia is an off-target toxicity in CD19/CD22-targeting CAR-T cells. HLH/MAS is relatively rare compared to the aforementioned toxicities and CAR-T cell therapy-related HLH/MAS usually happens secondary to CRS. As the ascending numbers of clinical trials in this field, more and more experiences have been summarized and instructions have been given to deal with these toxicities. Though a large amount of CAR-T trials have been put into practice in china, diagnosis and management of toxicities have not reached a consensus and unified norms are wanting. In our view, toxicities after CAR-T cell therapy are caused by systematic immune reactions and thus systematic administration and supportive care are needed as well as multidisciplinary coorperation. Glucocorticoid should be given after weighing pros and cons consciously. Patients with severe complications should be sent to intensive care unit (ICU) in order to acquire close monitor and timely treatment. Instructions from specialists are pivotal especially for those with neurological symptoms.

Infectious complications, immune reconstitution, and prophylactic strategies after chimeric antigen receptor T-cell therapy

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CD19-targeted chimeric antigen receptor (CAR) T cells have emerged as a breakthrough therapy providing an excellent remission rate and potential durable disease control for patients with relapse/refractory (R/R) hematologic malignancies. Besides impressive efficacy, CAR T cells harbor several potential side effects including immune-mediated toxicities, cytopenias, B cell aplasia and hypogammaglobulinemia. Infection has been increasingly recognized in patients treated with CAR T cell therapy. Several factors predispose these patients to infection including host-, CAR T- or malignancy-related factors. Fortunately, although studies have shown a high incidence of infection post CAR T cell, most infections were manageable. In contrast to patients who undergo hematopoietic stem cell transplant, little is known about post CAR T cell immune reconstitution. Therefore, evidence regarding prophylactic and vaccination strategies in these patients is still limited and most recommendations currently rely upon expert opinions. As CAR T cell therapy increasingly becomes the standard of care for R/R B lymphoid malignancies and may soon be applied in earlier lines of treatment or combined with other therapeutic modalities, immunocompromised states and infection after CAR T cell therapy will increasingly require clinical attention. Studies exploring infection and immune reconstitution after CAR T cell therapy are clinically relevant and will provide us better understanding on dynamics of immune function after CAR T cell therapy and insights into appropriate strategies for treatment/prophylaxis of infection in these patients.

HSCT in auto immune diseases: An update from the EBMT

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Autoimmune diseases (AD) are relatively common, affecting 5-8% of population, and although not all ADs are severe, some are very disabling, resistant to treatments and potentially life-threatening or life-shortening. For many patients, cure or sustained treatment free remission remains elusive and almost long-term therapy often comes with a burden of short-term and long-term side effects, including those from steroids or other immunosuppressive drugs. Additionally, costs of the disease are high both to individuals who frequently cannot work and society[1].

For four decades HSCT has been considered as “one-off” intensive means of long-term disease control, rather than chronic immunosuppression. Supported originally by animal models and serendipitous case reports, both autologous and allogeneic HSCT have been evolving as a specific clinical treatment as a treatment for severe, resistant ADs for over 25 years. An increasing evidence base has supported professional guidelines and an expanding clinical practice across a range of severe ADs[1,2,3].

The strongest evidence is for autologous HSCT in multiple sclerosis (MS)[4,5], and systemic sclerosis[6,7], for which autologous HSCT can be regarded as a standard of care based on randomised clinical trials. There is also evidence to support treatment of carefully selected patients with other neurological diseases, including neuromyelitis optica (NMO), chronic inflammatory demyelinating polyneuropathy (CIDP), myasthenia gravis, and stiff person syndrome[4], along with other rheumatological diseases, such as systemic lupus erythematosus and vasculitis, and gastrointestinal diseases, particularly Crohn’s disease[8]. Haematological immune cytopenias, type 1 diabetes and refractory coeliac disease have been rare indications[1-3].

Allogeneic HSCT has been largely restricted to paediatric patients, most commonly in immune cytopenias and juvenile idiopathic arthritis, and potentially provides long-term disease control in refractory AD. Syngeneic may be considered as alternative to autologous HSCT, if available. The EBMT retrospectively assessed long-term outcomes of allogeneic HSCT in 128 patients with various hematological and non-hematological severe ADs from 1997 to 2014[9]. Younger age, sex and more recent year of transplant were found to be significantly associated with improved outcomes on multivariate analysis. Compared with autologous HSCT, allogeneic HSCT can deliver the more radical option of complete replacement of a dysfunctional immune system, albeit with a greater risk of long-term complications, including GVHD. However, allogeneic HSCT may become increasingly more realistic in ADs with ongoing improvement in outcomes.

The EBMT Autoimmune Disease Working Party (ADWP) was founded in 1997 and has been central to many developments in the field, with its outputs supported by the EBMT registry[1,2,4,5,8,9,10]. As of June 2021, a total of 3614 HSCT procedures for ADs were reported to the EBMT registry since 1994; 3339 patients undergoing first autologous HSCT, with median age 38 years (3-76) and 202 patents undergoing allogeneic HSCT, with median age 11 years

(<1-64). Patients have been registered from 311 centres in 44 countries; 60% were female, 9% were <18 and 63 patients second or third HSCT procedures. Indications include 1780 patients with multiple sclerosis, 900 patients with connective tissue disorders, 191 patients with inflammatory arthritis, 62 patients with vasculitis, 222 patients with inflammatory bowel disease, 124 patients with immune cytopenias and 20 patients with type 1 diabetes, and 129 patients treated for other neurological diseases including CIDP (n=62), NMO (n=21) and myasthenia gravis (n=10). The predominant countries of activity were Italy, United Kingdom, Germany, Sweden, Spain, the Netherlands, Poland, France and Australia who made up around three quarters of the activity, although some other countries contributed highly based on per head of population. The Coronavirus disease-19 (COVID-19) pandemic has impacted on many aspects of HSCT practice, including for ADs where numbers of patients treated has been limited due to concerns of greater immunosuppression and risk of infection. Moving forwards, the EBMT have recently provided updated recommendations for best practice of HSCT in ADs to assist in the COVID-19 recovery phase[10].

Concurrent scientific studies continue to provide mechanistic insights to improve patient outcomes whilst increasing understanding of disease biology. There is reasonable evidence now HSCT as a means of delivering control of resistant inflammation through intensified cytotoxic treatment followed by ongoing altered immune reconstitution post-transplant (or 'immune re-boot')[1].

In surnmary, HSCT should be considered as an intensive treatment in carefully selected patients with severe, poor-prognosis and/or resistant ADs after multi-disciplinary discussion, weighing-up all options in accordance with EBMT and other professional guidelines. Interactions with professional societies, multi-disciplinary collaborations and health economic evaluations are essential to defining the place of HSCT in ADs, where there will always be a 'dynamic' with other evolving contemporary treatments. Ongoing clinical trials, registry analyses and biological studies are necessary to establish the best transplant regimen in each AD, elucidate mechanisms of action, develop clinical biomarkers to help select and monitor patients, and to determine health economic benefits and public health delivery across various AD indications. As more centers undertake procedures it is important to recognize that HSCT for AD presents unique challenges even to highly experienced HSCT teams, and shared knowledge and/or referral to 'centres of excellence' should be considered.

References

1. Alexander T, Greco R, Snowden JA. Hematopoietic Stem Cell Transplantation for Autoimmune Disease. *Annu Rev Med.* 2021;72:215-228.
2. Snowden JA, Badoglio M, Labopin M, et al. Evolution, trends, outcomes, and economics of hematopoietic stem cell transplantation in severe autoimmune diseases. *Blood Adv.* 2017;1:2742-2755.
3. Snowden JA, Saccardi R, Allez M, et al. Haematopoietic SCT in severe autoimmune diseases: updated guidelines of the European Group for Blood and Marrow Transplantation. *Bone Marrow Transplant.* 2012;47:770-790.
4. Burt RK, Balabanov R, Burman J, et al. Effect of Nonmyeloablative Hematopoietic Stem Cell Transplantation vs Continued Disease-Modifying Therapy on Disease Progression in Patients With Relapsing-Remitting Multiple Sclerosis: A Randomized Clinical Trial. *JAMA.* 2019;321:165-174

5. Sharrack B, Saccardi R, Alexander T, et al. Autologous haematopoietic stem cell transplantation and other cellular therapy in multiple sclerosis and immune-mediated neurological diseases: updated guidelines and recommendations from the EBMT Autoimmune Diseases Working Party (ADWP) and the Joint Accreditation Committee of EBMT and ISCT (JACIE). *Bone Marrow Transplant.* 2020;55:283-306
6. Farge D, Burt RK, Oliveira MC, et al. Cardiopulmonary assessment of patients with systemic sclerosis for hematopoietic stem cell transplantation: recommendations from the European Society for Blood and Marrow Transplantation Autoimmune Diseases Working Party and collaborating partners. *Bone Marrow Transplant.* 2017;52:1495-1503.
7. Sullivan KM, Goldmuntz EA, Keyes-Elstein L, et al. Myeloablative Autologous Stem-Cell Transplantation for Severe Scleroderma. *N Engl J Med.* 2018;378:35-47.
8. Snowden JA, Panes J, Alexander T, et al. Autologous Haematopoietic Stem Cell Transplantation (AHSCT) in Severe Crohn's Disease: A Review on Behalf of ECCO and EBMT. *J Crohns Colitis.* 2018;12:476-488.
9. Greco R, Labopin M, Badoglio M, et al. Allogeneic HSCT for Autoimmune Diseases: A Retrospective Study From the EBMT ADWP, IEWP, and PDWP Working Parties. *Front Immunol.* 2019;10:1570.
10. Greco R, Alexander T, Burman J, et al. Hematopoietic stem cell transplantation for autoimmune diseases in the time of COVID-19: EBMT guidelines and recommendations. *Bone Marrow Transplant.* 2021.

Acknowledgements
I acknowledge the input of Raffaella Greco, Chair EBMT ADWP, Milan, Italy, and Manuela Badoglio, EBMT Data Manager, Paris, France, in the writing of this article.

How to make accessible and affordable CAR T-cell with GMP facility

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Recently, a development of novel therapy beyond chemical products have been investigated for cancer treatment. Many groups of researchers have been studying utilization of human immune cells as biological drugs, so-called advanced therapy medicinal products, to eradicate malignant cells (1-5). An application of cellular immunotherapy, using various types of immune cells, might be categorized into native or modified cells. A genetically engineered technique modifying T lymphocyte to express chimeric antigen receptor (CAR) on its surface have been published and approved for the treatment of hematologic malignancies (6-9). The CAR is mainly comprised of three components, i.e., an extracellular antigen binding portion, a cytosolic signaling domain, and a segment between those parts, which may be consisted of a spacer, a transmembrane and/or a co-stimulatory domain(10). The extracellular portion is usually derived from a single-chain variable fragment of an antibody which specifically binds to tumor-associated antigen. The first approved biological drug is Tisagenlecleucel which is genetically modified autologous T lymphocytes expressing CAR against CD19 antigen (11). The CD19 antigen is a marker on B cell, ranging from precursor to mature stage; such antigen expresses on B-cell leukemia or lymphoma, which malignant clones are derived from B cell, as well as normal B cells. Development of the CD19-specific CAR is a prerequisite phase. The spacer, transmembrane and co-stimulatory domains need to carefully be chosen. CAR can be classified into several generations, mainly depending on a co-stimulatory domain. A first generation of CAR has no such domain, while second or third generations of CAR has one or two co-stimulatory domains respectively(10). Recently, the second generation of CAR is commonly used in a production of CAR-modified T lymphocyte (CAR T-cell) for clinical research and applications, for example, Tisagenlecleucel has a CD137 (4-1BB) as a co-stimulatory domain. Whereas a research team at Ramathibodi Hospital reported the production of a second generation of CAR T-cell contained CD28 as a co-stimulatory domain with various spacer lengths(12). Although second-generation CAR T-cells with different co-stimulatory domain could eradicate specific cancer cells. The CD28-contained CAR T-cells and the 4-1BB-contained CAR T-cells revealed to have different response pattern(13). The next step needed to be determined is a platform to transduce CAR into T lymphocyte. Most of clinical trials regarding CD19-specific CAR T-cells operated by viral transduction system, either retrovirus or lentivirus(6-9, 14). Lentiviral transduction system is claimed to be safer than retroviral system in term of generation of replication-competent viruses(15). Moreover, the self-inactivating lentiviral system has been developed to decrease activation of neighboring genes which may induce oncogenesis(16). The last step to develop CAR T cell is release tests which require some surveillant tests, e.g., viability, identity, potency, microbiological and stability tests(17). Production of CAR T-cell for a clinical application requires to comply with the good manufacturing practice (GMP) guideline (16, 17). The GMP guideline generally covers manufacturing processes of various products such as food or drugs. For CAR T-cell production, the main areas address in the GMP guideline include a production process, i.e., manufacturing, packaging, labelling and distributing, and other related issues, e.g., facility, personnel, equipment, quality control function, control of components, laboratory controls and recordkeeping. A major issue related to the production of CAR T-cell is a design of a facility. Since flows of materials, waste, staff and products require a decision whether unidirectional

or multidirectional flow. The multidirectional flow efficiently utilizes the area in the facility, while the unidirectional flow uses more space to separate dirty and clean corridors. Recently, CAR T-cell production facilities using the multidirectional model have been approved. For the manufacturing process, the traditional flask-based CAR T-cell production needs extensive labor work. Thus, the trend is to move towards bioreactors, such as the G-rex® (Wilson Wolf Manufacturing), the Xuri® (Cytiva), the Quatum® (Terumo) and the CliniMACS Prodigy® (Miltenyi Biotec). In particular, tisagenlecleucel manufacturing process transferred from an academic center to a pharmaceutical company needs process development and improvement(18). The manufacturing process starts with a frozen leukapheresis from a patient transported to a manufacturing facility and ends with a frozen modified T-cell product infused to that particular person. The manufacturing process of tisagenlecleucel mainly consists of two important steps, i.e., 1) T-cell activation and transduction and 2) modified T-cell expansion. Those steps are performed in bioreactors at two centralized facilities to meet a high demand of the modified T-cells. Whereas a group of researchers at Ramathibodi Hospital reported using simple plates and flasks for the production process(12). Recently, they have been using the G-rex® in a GMP compliant facility for the production of modified T cells for the reported case. Finally, those modified T-cell products need to meet releasing criteria, generally including safety, purity, identity, quantity and potency. The final modified T-cell product should not have any signals of infectious organisms, contain enough viable cells and CAR T-cells, which must show some degree of its potency against specific target cells. Since the cost of CAR T-cell product depends on the consumables related to bioreactors, determination of the T-cell expansion system needs to be carefully considered. A collaboration between an academic institute and a manufacturing company needs to be established to meet the high demand of this novel therapy.

References

1. Anurathapan U, Leen AM, Brenner MK, Vera JF. Engineered T cells for cancer treatment. Cytotherapy. 2014;16:713-33.
2. Kochenderfer JN, Dudley ME, Carpenter RO, Kassim SH, Rose JJ, Telford WG, et al. Donor-derived CD19-targeted T cells cause regression of malignancy persisting after allogeneic hematopoietic stem cell transplantation. Blood. 2013;122:4129-39.
3. Ritchie DS, Neeson PJ, Khot A, Peinert S, Tai T, Tainton K, et al. Persistence and efficacy of second generation CAR T cell against the LeY antigen in acute myeloid leukemia. Mol Ther. 2013;21:2122-9.
4. Savoldo B, Rooney CM, Di Stasi A, Abken H, Hombach A, Foster AE, et al. Epstein Barr virus specific cytotoxic T lymphocytes expressing the anti-CD30zeta artificial chimeric T-cell receptor for immunotherapy of Hodgkin disease. Blood. 2007;110:2620-30.
5. Shaffer DR, Savoldo B, Yi Z, Chow KK, Kakarla S, Spencer DM, et al. T cells redirected against CD70 for the immunotherapy of CD70-positive malignancies. Blood. 2011;117:4304-14.
6. Maude SL, Laetsch TW, Buechner J, Rives S, Boyer M, Bittencourt H, et al. Tisagenlecleucel in Children and Young Adults with B-Cell Lymphoblastic Leukemia. N Engl J Med. 2018;378:439-48.
7. Locke FL, Ghobadi A, Jacobson CA, Miklos DB, Lekakis LJ, Oluwole OO, et al. Long-term safety and activity of axicabtagene ciloleucel in refractory large B-cell lymphoma (ZUMA-1): a single-arm, multicentre, phase 1-2 trial. Lancet Oncol. 2019;20:31-42.
8. Raje N, Berdeja J, Lin Y, Siegel D, Jagannath S, Madduri D, et al. Anti-BCMA CAR T-Cell Therapy bb2121 in Relapsed or Refractory Multiple Myeloma. N Engl J Med. 2019;380:1726-37.
9. Abramson JS, Palomba ML, Gordon LI, Lunning MA, Wang M, Arnason J, et al. Lisocabtagene maraleucel for patients with relapsed or refractory large B-cell lymphomas (TRANSCEND NHL 001): a multicentre seamless design study. Lancet. 2020;396:839-52.

10. Luo Y, Song G, Liang S, Li F, Liu K. Research advances in chimeric antigen receptor-modified T-cell therapy (Review). Exp Ther Med. 2021;21:484.
11. Grupp SA, Kalos M, Barrett D, Aplenc R, Porter DL, Rheingold SR, et al. Chimeric antigen receptor-modified T cells for acute lymphoid leukemia. N Engl J Med. 2013;368:1509-18.
12. Prasongtanakij S, Anurathapan U, Vanichapol T, Jittorntrum B, Atjanasuppat K, Pongpitcha P, et al. Production and characterization of haploidentical CD19 CAR T cells: Validated to induce a continuous complete remission in a patient with relapsed refractory B-cell ALL. Asia Pac J Clin Oncol. 2020.
13. Li S, Zhang J, Wang M, Fu G, Li Y, Pei L, et al. Treatment of acute lymphoblastic leukaemia with the second generation of CD19 CAR-T containing either CD28 or 4-1BB. Br J Haematol. 2018;181:360-71.
14. Ramos CA, Savoldo B, Dotti G. CD19-CAR trials. Cancer J. 2014;20:112-8.
15. Cooray S, Howe SJ, Thrasher AJ. Retrovirus and lentivirus vector design and methods of cell conditioning. Methods Enzymol. 2012;507:29-57.
16. Poorebrahim M, Sadeghi S, Fakhr E, Abazari MF, Poortahmasebi V, Kheirollahi A, et al. Production of CAR T-cells by GMP-grade lentiviral vectors: latest advances and future prospects. Crit Rev Clin Lab Sci. 2019;56:393-419.
17. Gee AP. GMP CAR-T cell production. Best Pract Res Clin Haematol. 2018;31:126-34.
18. Tyagarajan S, Spencer T, Smith J. Optimizing CAR-T Cell Manufacturing Processes during Pivotal Clinical Trials. Mol Ther Methods Clin Dev. 2020;16:136-44.

Non-viral based CAR-T cell manufacturing and clinical study for leukemia and Lymphoma

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The *piggyBac* transposon system is a promising genetic tool for stable, non-viral gene engineering of primary human T cells. We have improved these process and construct to avoid magnetic cell isolation, shorten the culture period, and increase *in vivo* efficacy to provide clinical grade CD19.CAR T cells. The major subset of CAR-T cells was phenotypically CD8+CD45RA+CCR7+, closely related T-memory stem cells. In 7 days coculture assay, CD19.CAR-T cells mostly eliminated CD19 positive tumor cell lines at an E:T ratio of 1:5. In NSG mice inoculated with CD19 positive tumor cells, CD19.CAR-T cells dramatically inhibit tumor growth. High throughput sequencing to examine the *CAR* integration pattern revealed the frequency of integration within 50 kb of transcription starting site of proto-oncogene of *piggybac* was comparable with that of retrovirus (3.9% vs 5.1%, p=0.163) while less than that of lentivirus (3.9% vs 5.7%, p=0.0219). These preclinical data support a *piggyBac* transposon technology as cost-effective and safe therapeutic platform for CD19.CAR-T cell therapy for the clinical trial. A phase I clinical trial of *piggyBac* transposon - based CD19.CAR-T cells for acute lymphoblastic leukemia is underway in Japan (UMIN000030984). We are also collaborating to develop a piggyBac transposon mediated CAR-T cells with Chulalongkorn University in Thailand. Finally, the first patient with non-Hodgkin lymphoma was treated with piggyBac mediated CAR-T cells in Chulalongkorn University Hospital in October 2020.

HSCT as an option for monogenic immunodysregulatory and autoinflammatory disorders: considerations, challenges and opportunities

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Inborn errors of immunity (IEI) are a group of rare genetic disorder of the immune system. Currently, over 400 genetic defects have been identified. Patients may present with a variety of manifestations including susceptibility to infections, inflammation, autoimmunity, allergy and malignancy. The identification of specific genetic defects and pathway alterations enable targeted therapy, such as monoclonal antibodies, cytokines and small molecule inhibitors, to be used for immunodysregulatory and autoinflammatory disorders. At the same time, modern approaches in HSCT and improved supportive care provide opportunities of cure for this group of patients. Patient counseling for lifelong immune modulation versus a definitive corrective procedure using HSCT is becoming increasingly challenging, when compared with a relatively straightforward decision-making for more ‘classical’ forms of primary immunodeficiencies such as severe combined immunodeficiency, Wiskott-Aldrich syndrome, chronic granulomatous disease, leukocyte adhesion deficiency, primary HLH etc. In this talk, the speaker will discuss the general principles of management for IEI, and highlight aspects of immune defects that can / cannot be corrected by replacement of the haematopoietic component. Factors that need to be considered when making decisions for HSCT / conservative therapy will be elaborated, including an understanding of the disease pathogenetic mechanisms, genotype and phenotype correlation, drug availability and cost, comorbidities, donor options, implication on long-term health outcomes and economic considerations.

Update on treatment of chronic GVHD

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Chronic graft-versus-host disease (GVHD) occurs in approximately 40% of patients who undergo allogeneic hematopoietic cell transplantation. Chronic GVHD affects a variety of organs and causes significant morbidity and mortality. Manifestations of chronic GVHD resemble those of autoimmune diseases. Inflammation, cellular immunity, humoral immunity and fibrosis are implicated with pathogenesis. The 2005 NIH consensus criteria for chronic GVHD have set standards for designing and reporting clinical trials, and the criteria were revised in 2014 incorporating accumulated evidences and controversies. In addition, preclinical experiments of chronic GVHD have revealed the central roles of regulatory T cells, B-cell signaling, Th17 cells, Tc17 cells, follicular helper T cells, follicular regulatory T cells, and fibrosis-promoting factors. All these efforts led to the first approval of ibrutinib for treatment of chronic GVHD after failure of one or more lines of systemic therapy, and an increasing number of investigational agents that target different biological pathways of chronic GVHD are under development in clinical trials. To address challenges in a rapidly changing field, a third NIH consensus project was held in 2020, and investigators aimed to define basic and clinical research directions that may lead to significant change in chronic GVHD management over the next 5 years. In this talk, highlights of the 2020 NIH consensus, characteristics of chronic GVHD in Japanese patients, and the results of recent randomized studies of ibrutinib, ruxolitinib and belumosudil for initial and subsequent-lines of treatment for chronic GVHD are discussed.

Novel drugs for GVHD

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Allogeneic hematopoietic cell transplantation (allo-HCT) is the only curative approach for a large group of hematological malignancies but its success is hampered by acute and chronic graft-versus-host disease (GVHD). Acute GVHD is a major cause of death following allo-HCT and for several decades little progress has been made in treating patients, with corticosteroids being the mainstay of therapy. In the last 5 years, multiple novel therapy approaches for GVHD have entered the arena.

In 2020 a large randomized phase III trials for acute GVHD reported that ruxolitinib was superior to best available therapy for acute GVHD (REACH2 trial). In 2021 the REACH3 trial showed that ruxolitinib was superior to best available therapy for chronic GVHD (REACH3 trial). Besides the treatment strategies that rely on kinase inhibition (REACH and GRAVITAS trials) novel regenerative approaches (IL-22, R-spondin, glucogon like peptide GLP-2) are under intensive investigation. Also GVHD prevention by Abatacept, Dipeptidyl Peptidase 4 Inhibition and post transplant cyclophosphamide are very promising strategies which need further evaluation.

In my talk I will present these therapies as well as the underlying mechanisms that contribute to the clinical picture of acute GVHD.

Mesenchymal stem cell (MSC) as a therapy for GvHD

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MSC has potent immunosuppressive function and enhances regeneration, so it is ideal for GvHD setting. However, despite the initial encouraging results, subsequent multicenter trials failed to show any significant benefit. There are many postulations for the failures. One of them is the biological differences of MSC derived from different sources and donors. The limited number of ex vivo culture passage also affects the application feasibility. To overcome such quality control issue of heterogeneity and passage effects, we converted MSC into induced pluripotent stem cells (iPSC) and then differentiated them back to MSC (Patent filed). Such approach can produce a relatively unlimited supply of homogeneous MSC with same origin. Another factor affecting the efficacy of MSC therapy is the discrepancy of immunosuppressive function from different cell origin. We found that MSC derived from adipose tissue has the highest immunosuppressive potential when compared to umbilical cord or bone marrow. We identified possible mechanism to explain such variation. By comparing the RNAseq profiles of MSC from the 3 tissues, we identified the potential molecule involved. Immunological tests confirmed such association. Furthermore, we discovered some existing drugs that can enhance the expression of this molecule and render MSC more immunosuppressive. We also explored the use of MSC derived exosomes as a substitute of MSC to improve product consistency. Finally for the product scaling up problem, we invented an ex vivo automated oscillating cylinder culture system using microbeads that can improve the yield of MSC. All these helps to move MSC therapy back to the bedside again.

Graft manipulation in haploidentical HSCT

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To overcome of HLA barrier and prevent GVHD, several strategies has been applied in haploidentical hematopoietic cell transplantation (haplo-HSCT). Graft manipulation has been used for sustained stable engraftment and acceptable incidence of GVHD in haplo-HSCT. Currently, allogeneic HSCT using an HLA-haploidentical family donor (HFD) is considered an accepted treatment option for patients who cannot find a matched related donor (MRD) or unrelated donor (URD). Depletion of $\alpha\beta^+$ T cells from the graft is the most recent approach as an ex vivo T cell depletion flatform [1-3]. Depletion of $\alpha\beta^+$ T cells produced grafts containing many $\gamma\delta^+$ lymphocytes as well as other effector cells. While $\alpha\beta^+$ T cells are known to be associated with the initiation of GVHD, $\gamma\delta^+$ T cells can enhance immune reconstitution and are not implicated in development of GVHD [4].

Our experience on the depletion technique targeting $\alpha\beta^+$ T cells revealed the median log depletion of 4.0 with median recovery rates of CD34+, NK, and $\gamma\delta^+$ T cells at 90.4%, 74.9%, and 75.9%, respectively [5]. The efficacy of depletion of $\alpha\beta^+$ T cells has significantly improved over time with most of recent log depletion well over 4.0 consistently.

At our center, patients with malignant or non-malignant disease received HSCT from HFD using αβ+ T cell-depleted graft. In our recent study on pediatric patients with AML, the outcomes of HSCT from HFD were comparable with those of HSCT from MSD or URD. Sixty-seven pediatric patients with AML in CR or PR underwent HSCT at Asan Medical Center Children's Hospital (AMCCH) between November 2011 and December 2020. Eleven patients received HSCT from MSD, 29 from URD and 27 from HFD. For MSD or URD, myeloablative conditioning regimen was used which consisted of busulfan (16mg/kg) and cyclophosphamide (120 mg/kg) ± ATG (4 or 7.5 mg/kg). For HFD, reduced-intensity conditioning regimen was used which included TBI (600 cGy), fludarabine (180 mg/m2), cyclophosphamide (100 mg/kg) and ATG (3 mg/kg). The Leukemia-free survival of HSCT-MFD was comparable to HSCT-MSD/URD (77.2% vs 70.5%, P=0.865). We also reported the HSCT for pediatric patients with myelodysplastic syndrome (MDS) [6]. Of 36 enrolled patients with MDS who received HSCT at AMCCH between 1997 and 2007, 9 patients received HSCT from HFD with OS of 86%. Another study at our center reported on the outcome of HSCT for 67 pediatric patients with acquired aplastic anemia (14 MSD, 21 URD and 32 HFD) [7]. Neutrophil engraftment significantly faster in haplo-HSCT compared with HSCT from other donors (*P*=.022). OS was similar regardless of donor types (MSD 92.9%, URD 95.2% and HFD 93.4%, *P*=.432). This study demonstrated that optimized haploidentical transplantation using selective T cell depletion and conditioning regimens including low-dose total body irradiation for enhancing engraftment may be a realistic therapeutic option for pediatric patients with severe aplastic anemia.

Our clinical experiences suggest that HSCT using $\alpha\beta^+$ T cell-depleted graft is feasible in terms of engraftment rate and incidence of GVHD and TRM. However, delayed immune reconstitution after haplo-HSCT is a major issue to be improved further as the prolonged impaired immunity increases the risk infections. Viral infections refractory to antiviral therapy are a life-threatening complication following allogeneic HSCT. Depletion of alloreactive $CD45RA^+$ naïve T cells produce the graft containing abundant pathogen-specific memory T

cells which can be used as a therapeutic donor lymphocyte infusion (DLI) to restore protective T-cell immunity in patients with refractory viral infections after allogeneic HSCT [8]. At our center, 17 depletion procedures targeting $CD45RA^+$ T cells using CliniMACS were performed between May 2017 and January 2021. Overall $CD3^+$ T cells were depleted at a median of 0.8 log (range, 0.4–1.3) with > 99% purity of $CD45RO^+$ T cells. CD45RA depletion procedure resulted in 2.7–5.0 log (median 3.4) reduction of $CD45RA^+$ T cells. Donor B cells were completely removed with a median depletion of 4.3 log and significant proportion of NK cells was also depleted with a median depletion of 2.9 log. Eight patients (3 hematologic malignancy, 4 SAA, 1 PID) with refractory viral infections (6 CMV, 1 ADV and 1 BKV) after haplo-HSCT received CD45RA-depleted DLI. Eight patients received their first CD45RA-depleted DLI at a median of 112 days (range, 66–266) post-HSCT at a dose of 2.5×10^4 $CD3^+$ cells/kg from their original donor with the median number of 3 (range, 1–12) DLIs. Patient received a subsequent DLI of thawed cells at escalated dose from 5×10^4 to 5×10^5 $CD3^+$ cells/kg. Five of 8 evaluable patients cleared their viremia at a median of 67 days (range, 53–90) after first DLI. Of 8 patients, only 1 patient developed grade 1 acute skin GVHD at D+58 after DLI, and none had chronic GVHD. With a median follow-up of 19 months (range, 4–46), four patients survived and the remaining 4 died (1 CMV pneumonia, 1 CMV encephalitis, 1 BKV encephalitis and 1 TMA). This CD45RA depletion procedure is highly efficient to remove alloreactive naïve T cells while preserving $CD45RO^+$ memory T cells. Our study suggested that CD45RA-depleted DLI is feasible with a low risk of GVHD. Therefore, infusion of naïve T cell-depleted graft should be offered in patients with refractory viral infection after haploidentical HSCT.

Graft manipulation targeting $\alpha\beta^+$ T cells and $CD45RA^+$ T cells has significantly improved the outcomes of allogeneic HSCT from HFD providing an emerging evidence of haplo-HSCT as an attractive treatment option for pediatric patients without an optimal related or unrelated donor.

References

1. Lang P, Feuchtinger T, Teltschik HM, et al. Improved immune recovery after transplantatio n of TCRalphabeta/CD19-depleted allografts from haploidentical donors in pediatric patients. Bone Marrow Transplant 2015;50 Suppl 2:S6-10. DOI: 10.1038/bmt.2015.87.
2. Im HJ, Koh KN, Suh JK, et al. Haploidentical HCT using an alphabeta T-cell-depleted graft with targeted alphabeta(+) cells by add-back after a reduced intensity preparative regimen containing low-dose TBI. Bone Marrow Transplant 2016;51(9):1217-22. DOI: 10.1038/bmt.20 16.114.
3. Locatelli F, Merli P, Pagliara D, et al. Outcome of children with acute leukemia given HLA-haploidentical HSCT after alphabeta T-cell and B-cell depletion. Blood 2017;130(5):677-685. DOI: 10.1182/blood-2017-04-779769.
4. Minculescu L, Sengelov H. The role of gamma delta T cells in haematopoietic stem cell tr ansplantation. Scand J Immunol 2015;81(6):459-68. DOI: 10.1111/sji.12289.
5. Choi ES, Im HJ, Kim H, et al. Depletion of alphabeta(+) T cells for a haploidentical hemat opoietic stem cell transplantation in children. J Clin Apher 2018;33(4):521-528. DOI: 10.100 2/jca.21634.
6. Yoo JW, Im HJ, Kim H, et al. Improved outcomes of allogeneic hematopoietic stem cell tr ansplantation including haploidentical transplantation for childhood myelodysplastic syndrom e. Bone Marrow Transplant 2020;55(8):1595-1603. DOI: 10.1038/s41409-020-0814-8.
7. Kim H, Im HJ, Koh KN, et al. Comparable Outcome with a Faster Engraftment of Optimiz ed Haploidentical Hematopoietic Stem Cell Transplantation Compared with Transplantations from Other Donor Types in Pediatric Acquired Aplastic Anemia. Biol Blood Marrow Transpl ant 2019;25(5):965-974. DOI: 10.1016/j.bbmt.2019.01.010.

8. Castagna L, Valli V, Timofeeva I, et al. Feasibility and Efficacy of CD45RA+ Depleted Don or Lymphocytes Infusion After Haploidentical Transplantation With Post-Transplantation Cycl ophosphamide in Patients With Hematological Malignancies. Transplant Cell Ther 2021;27 (6):478 e1-478 e5. DOI: 10.1016/j.jtct.2021.03.010.

CAR T-cell therapy for patients with multiple myeloma

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Multiple myeloma (MM) is a malignancy of plasma cells originating in the bone marrow. Overthe past two decades, the availability of proteasome inhibitors, immunomodulatory agents and anti-CD38 monoclonal antibodies has increased the median survival duration of patients with MM from3 years to 6 years from initial diagnosis. However, MM remains largely incurable. Chimeric antigen receptor (CAR) T-cell therapy is one of the rapidly emerging and highly promising immunotherapeutic options that has shown unprecedented results in MM. CAR T cell therapy worksby mechanisms distinct from those of other MM therapies and involves the modification of patientor donor T cells to target specific cell-surface antigens, attacking tumor cells without the physiological need for HLA-presentation. CARs are artificial fusion proteins, consist of an antigen-recognition domain, an activation domain, and co-stimulatory domains. Many CAR T cell productshave been tested in clinical trials have demonstrated that patients with relapsed and/or refractory MM (RRMM) can achieve objective responses.

The most important determinant of the success of CAR T cell therapy is the choice of the targetantigen. Of the antigens identified so far, B cell maturation antigen (BCMA), a member of the TNFsuperfamily, has the most favorable expression pattern for a MM cell-directed CAR, which is expressed on the plasma cells but not on hematopoietic stem cells, and promotes growth and proliferation of plasma cells. The first-in-human Phase I clinical trial to test the efficacy of the BCMA targeted CAR T cells in RRMM (NCT02215967) conducted by US National Cancer Institute (NCI) in 2013. The overall response rate (ORR) was 81%, with 63% demonstrating very good partial (VGPR) or complete response (CR) [2]. In 2017 a breakthrough in CAR T cell development for myeloma was reached, bb2121 (Bluebird Bio) heralded as the latest front-runner for FDA's approval for MM treatment, ORR was 73% with significantly improved PFS (median PFS 8.8 months) in the RRMM patients [3]. At 2019 ASH, we firstly report 46 patients that had been infusedwith this intended dose of the autologous BCMA CAR-T cells, and 44 patients had reached at least1 month of follow-up [4]. As of this data cut-off, the ORR for the 44 evaluable patients was 79.6%, including 2 sCRs, 16 CRs, 8 VGPRs and 8 PRs, and 16 patients reached MRD-negative response. The CAR-T cell expansion and persistence were consistently observed throughout these patients. The median PFS is 15 months, and the median OS result has not been reached (49.16% PFS, and 53.95% OS at 24 months) [4, 5]. At 2021, reported 18 patients with RRMM that had been infused with fully human BCMA-specific CAR, CT103A, the ORR 100%, with CR or sCR 72.2%. At 1 year, the progression-free survival rate was 58.3% for all cohorts and 79.1% for the patients without extramedullary myeloma [6].

CD19 is typically absent in matured plasma cells, minor subsets of myeloma cells with uniqueproperties express low CD19, associated with drug resistance and relapse-promoting properties. Comparing the durability of responses will be important to assess the efficacy of BCMA-CD19- CAR T and CD19-CAR T cells. We have recently reported that BCMA-CD19 dual FasT CAR-T GC012F showed early, deep and durable responses with a high ORR (94.7% - VGPR and better) including a high MRD-sCR rate (DL3=100%, n=9) in high risk RRMM pts including those refractory to anti-CD38, PI and IMIDs with a favorable safety profile, which

was sustained with a median duration of follow up of 7.3 months at cut off [5, 7].

Although BCMA CAR-T is very promising therapy, it has its own set of challenges. Toxicities from BCMA CAR T cell therapy are frequent events. The most common sign of CRS is fever, which can also manifest as nausea, flu, hypotension, hypoxia and other abnormalities. Neurological toxicities are another important adverse effect. High-grade toxicities correlated with heavy tumor burden and high CAR T-cell dosage. Toxicities are typically managed with drugs such as IL6R- antagonist for CRS, and corticosteroids for neurologic symptoms. BCMA antigen escape occurs, often lose its expression upon disease relapse after the first CAR T infusion. Previous study demonstrated the existence of T cell reactivity against the mouse scFv, consistent with previous reports of an immune response against anti-CD19 CARs containing mouse components and the detection of anti-mouse antibodies in patients with MM in another study. Several strategies are being pursued to address these challenges, including the use of γ-secretase inhibitors to enhance BCMA molecule density on MM cells and reduce the amount of soluble BCMA in serum and use of CAR products with defined T-cell subset compositions and humanized targeting domains to reduce immunogenicity and promote engraftment and in vivo expansion. Chun R Tong had reported human CAR-T could overcome immunogenicity and induced CR in patients who had failed murine CD19 CAR T therapy. And the CRS were mild, without treatment-related deaths [8].

Besides BCMA, various other antigens have been explored for a MM cell-directed CAR, one of the well-studied-antigen is signaling lymphocytic activation molecule 7 (SLAMF7). The CARAMBA clinical trial, using a novel SLAMF7 CAR T-cell model utilizing non-viral gene transfer approach of the Sleeping Beauty transposon system, investigates the feasibility, safety, and anti-myeloma efficacy of autologous SLAMF7 CAR-T cells [9].

CAR T-cell therapy is a great advance against MM, therefore, improved understanding of the pathophysiology of these processes will aid in the development of optimum strategies of immunosuppression and supportive care. Longer follow-up of the current studies and results from larger ongoing studies will provide new insights in the near future.

Reference

1. Timmers, M. et al. Chimeric antigen receptor-modified T cell therapy in multiple myeloma: beyond B cell maturation antigen. Front. Immunol. 2019; 10: 1613.
2. Ali, S. A. et al. T cells expressing an anti-B-cell maturation antigen chimeric antigen receptor cause remissions of multiple myeloma. Blood. 2016; 128: 1688–700.
3. Raje, N. et al. Anti-BCMA CAR T-cell therapy bb2121 in relapsed or refractory multiple myeloma. New Engl. J. Med. 2019; 380: 1726–37.
4. Weijun Fu, et al. Efficacy and Safety of CAR-T Therapy with Safety Switch Targeting Bcma for Patients with Relapsed/Refractory Multiple Myeloma in a Phase 1 Clinical Study. Blood. 2019; 134: 3154.
5. Hua Jiang et al. Clinical Results of a Multicenter Study of the First-in-Human Dual BCMA and CD19 Targeted Novel Platform Fast CAR-T Cell Therapy for Patients with Relapsed/Refractory Multiple Myeloma. Blood. 2020; 136: 25-6.
6. Di Wang, et al. A phase 1 study of a novel fully human BCMA-targeting CAR (CT103A) in patients with relapsed/refractory multiple myeloma. Blood. 2021; 137: 2890–2901.
7. Juan Du et al. Long-term Follow-up Results of a Multicenter First-in-Human Study of the Dual BCMA/CD19 Targeted FasT CAR-T GC012F for Patients with Relapsed/Refractory Multiple Myeloma. EHA. 2021; 325720; EP962.
8. Chun R Tong et al. The Salvage Treatment of Humanized-CD19 CAR-T Cells for Relapsed B-ALL After Prior Murinized-CD19 CAR-T Cell Therapy. EHA. 2021; 325100; EP346.
9. Sabrina et al. CARAMBA: a first-in-human clinical trial with SLAMF7 CAR-T cells prepared by virus-

free Sleeping Beauty gene transfer to treat multiple myeloma. Gene Ther. 2021; Apr13. doi.org/10.1038/s41434-021-00254-w.

Management of Relapsed Lymphoma Before and After CAR-T cell infusion

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Chimeric antigen receptor (CAR) T-cell therapy is highly effective in the treatment of B-cell acute lymphoblastic leukemia (ALL) and B-cell lymphoma, providing alternative therapeutic options for patients who have failed conventional treatment and are refractory to chemotherapy.[1–3] Moreover, with more emerging data in the literature, CAR-T cell therapy can serve as a bridge to other therapeutic strategies and improve patient prognosis.[4]

At our Centre, Singapore General Hospital (SGH), Department of Haematology, we see about 80-130 cases of lymphoma annually where the majority of cases are diffuse large B cell lymphoma (DLBCL). Based on statistics, majority of patients with DLBCL will have a complete response (60%) but 30% will relapse and approximately 30% of these patients will be chemo-refractory and would not benefit from high dose chemotherapy and an autologous stem cell transplantation. These patients have a dismal prognosis and die from disease progression. CAR-T provides an alternative therapeutic option for these patients.

In Singapore, Tisagenlecleucel is commercially available and our centre at SGH is FACT JACIE accredited to administer cell therapy products.[5] I will present an overview of patients with relapsed/refractory diffuse large B cell lymphoma referred to our centre for consideration of CAR-T cell therapy and their subsequent management.

Figure 1. The Roadmap of the CAR-T cell Therapy Program at SGH.

Referred patients meeting the indications for Tisagenlecleucel will undergo a clinical consultation with a haematologist. During the consultation, the patient will be counselled about CAR-T cell therapy and undergo fitness assessment based on performance status and organ function with particular focus on the cardiac, respiratory, renal and hepatic function. Patients will undergo disease re-assessment and staging if not done recently and this would involve a repeat LDH, PET CT and biopsy. At our centre, we will perform immuno-histochemical staining on the biopsy for CD19. If CD19 is dim or absent then we would not recommend the patient to undergo anti-CD19 CAR-T cell therapy. The patient will also undergo financial counselling. Once the patient is assessed to be fit and eligible for CAR T cell therapy, the case must be presented at the MDT cell therapy and haematopoietic stem cell transplantation to obtain consensus and this is part of our clinical governance program.

The critical management of these patients before CAR T cell infusion is really a logistical one. Often the patients who come for CAR T cell therapy are heavily pre-treated and possibly post autologous stem cell transplantation. There are several hurdles in such patients. Firstly, the process of leukapheresis; in such heavily pre-treated patients, they may not have much circulating lymphocytes and the state of “health” of these lymphocytes may not be ideal and this will affect the manufacturing of CAR T cells. Secondly, the lymphoma disease

characteristics and patient factors need to be considered; if the disease is bulky and aggressive, patients may not be able to tolerate the leukapheresis and having to be off certain treatment to accommodate the wash-put period of these drugs before leukapheresis is a challenge in itself. With experience and based on real world data, it is recommended that patients should have a performance status of 2 or less and that the patient must have a prognosis of at least 3 months or more before being eligible for CAR T cell therapy. Once the collection of mono-nuclear cells is completed we then need to determine if patient requires bridging therapy and if so, what would be a sensible bridge while the CAR-T cells are being manufactured.[6,7] This bridging period can be between 5-6 weeks.

At our centre, patients undergo CAR T-cell therapy as an inpatient. They undergo the standard lymphodepletion therapy with cyclophosphamide and fludarabine followed by the CAR T-cell therapy product. Patients are then monitored for cytokine release syndrome (CRS) and immune cell therapy associated neurotoxicity syndrome (ICANS). We adhere to the consensus grading of CRS and ICANS[8] and have SOPs in place to manage these toxicities as per the EBMT standards and recommendations. Similar to what has been published, severity of CRS is often related to disease burden and the more severe the CRS, the increased risk of developing ICANS. Often these toxicities are self-limiting and are managed with tocilizumab, corticosteroids and supportive measures.[9,10] Another recognised toxicity are cytopenia not related to the LD regimen or the disease where patients develop prolonged neutropenia and thrombocytopenia.[11]

Once patient is well with resolution of CRS and/or ICANS and cytopenia improved or stable, they are discharged and seen in clinic weekly for 1 month then monthly if well. They remain on anti-viral prophylaxis with acyclovir, antibiotics with ciprofloxacin if neutropenic and co-trimoxazole according to EBMT recommendations.[11] Patients undergo disease assessment at 1 month with a PET CT and if bone marrow was involved, a repeat bone marrow biopsy. They continue to have their FBC and LDH monitored and have PET CT done at 3 monthly interval if in CR or at closer intervals if in PR at 1 month.

Conclusion

Despite having received CAR-T cell therapy, 30–60%[1-3] of patients will relapse. Some are CD19+ve relapses, probably due to poor persistence of CAR T-cells which is likely multifactorial, while others are CD19-ve relapses due to antigen escape or downregulation of the CD19 antigen. These relapse mechanisms pose a great challenge for disease control. Therefore, optimising patient selection and understanding the mechanisms driving post-CAR relapses and establishing preventative and treatment algorithms is the next important step to take.

References

1. S.L. Maude, T.W. Laetsch, J. Buechner, S. Rives, M. Boyer HB, P. Bader, M.R. Verneris, H.E. Stefanski, G.D. Myers, M. Qayed, B. De Moerloose, H. Hiramatsu, K. Schlis, K.L. Davis, P.L. Martin, E.R. Nemecek, G.A. Yanik, C. Peters, A. Baruchel, N. Boissel, F. Mechinaud, A. Balduzzi JK, C.H. June, B.L. Levine, P. Wood, T. Taran, M. Leung, K.T. Mueller, Y. Zhang, K. Sen, D. Lebwohl, M.A. Pulsipher and SAG. Tisagenlecleucel in Children and Young Adults with B-Cell Lymphoblastic Leukemia | Enhanced Reader. [cited 2021 Jan 14];Available from: chrome-extension://dagcmkpagjlhakfdhnbomgmjdpkdklff/enhanced-

reader.html?openApp&pdf=https%3A%2F%2Fwww.nejm.org%2Fdoi%2Fpdf%2F10.1056%2FNEJMoa1709866%3FarticleTools%3Dtrue

2. Stephen J. Schuster, M.D., Michael R. Bishop, M.D., Constantine S. Tam MD, Edmund K. Waller, M.D., Ph.D., Peter Borchmann, M.D., Joseph P. McGuirk, D.O., Ulrich Jäger, M.D., Samantha Jaglowski, M.D., Charalambos Andreadis, M.D., Jason R. Westin, M.D., Isabelle Fleury, M.D., Veronika Bachanova, M.D., Ph.D., S. Ronan Foley, M.D., PD, Lida B. Pacaud, M.D., Rakesh Awasthi, Ph.D., Jufen Chu, Ph.D., Özlem Anak, M.D., Gilles Salles, M.D., Ph.D., and Richard T. Maziarz, M.D. for the JI. Tisagenlecleucel in Adult Relapsed or Refractory Diffuse Large B-Cell Lymphoma | Enhanced Reader [Internet]. [cited 2021 Jan 14];Available from: chrome-extension://dagcmkpagjlhakfdhnbomgmjdpkdklff/enhanced-reader.html?openApp&pdf=https%3A%2F%2Fwww.nejm.org%2Fdoi%2Fpdf%2F10.1056%2FNEJMoa1804980%3FarticleTools%3Dtrue
3. Whittington MD, McQueen RB, Ollendorf DA, et al. Long-term Survival and Cost-effectiveness Associated With Axicabtagene Ciloleucel vs Chemotherapy for Treatment of B-Cell Lymphoma. JAMA Netw open 2019;2(2):e190035.
4. T ZYcHsYtXzYlXlZyFjMgZw. Chimeric antigen receptor T-cell therapy as a bridge to haematopoietic stem cell transplantation for refractory/ relapsed B-cell acute lymphoblastic leukemia.
5. Maus M V., Nikiforow S. The Why, what, and How of the New FACT standards for immune effector cells [Internet]. J. Immunother. Cancer. 2017 [cited 2020 Jun 4];5(1):36. Available from: http://jitc.bmj.com/lookup/doi/10.1186/s40425-017-0239-0
6. Perica K, Flynn J, Curran KJ, et al. Impact of bridging chemotherapy on clinical outcome of CD19 CAR T therapy in adult acute lymphoblastic leukemia. Leukemia [Internet] 2021 [cited 2021 Mar 15];Available from: http://www.nature.com/articles/s41375-021-01196-3
7. Pinnix CC, Gunther JR, Dabaja BS, et al. Bridging therapy prior to axicabtagene ciloleucel for relapsed/refractory large B-cell lymphoma. Blood Adv 2020;4(13):2871–83.
8. Lee DW, Santomasso BD, Locke FL, et al. ASTCT Consensus Grading for Cytokine Release Syndrome and Neurologic Toxicity Associated with Immune Effector Cells-NC-ND license. (http://creativecommons.org/licenses/by-nc-nd/4.0/). 2019 [cited 2021 Jan 13];Available from: https://doi.org/10.1016/j.bbmt.2018.12.758
9. Locke FL, Ghobadi A, Jacobson CA, et al. Long-term safety and activity of axicabtagene ciloleucel in refractory large B-cell lymphoma (ZUMA-1): a single-arm, multicentre, phase 1-2 trial. Lancet Oncol [Internet] 2019 [cited 2020 Mar 2];20(1):31–42. Available from: http://www.ncbi.nlm.nih.gov/pubmed/30518502
10. Awasthi R, Pacaud L, Waldron E, et al. Tisagenlecleucel cellular kinetics, dose, and immunogenicity in relation to clinical factors in relapsed/refractory DLBCL. Blood Adv [Internet] 2020 [cited 2020 Mar 2];4(3):560–72. Available from: http://www.ncbi.nlm.nih.gov/pubmed/32045475
11. Yakoub-Agha I, Chabannon C, Bader P, et al. Management of adults and children undergoing chimeric antigen receptor T-cell therapy: Best practice recommendations of the European Society for Blood and Marrow Transplantation (EBMT) and the Joint Accreditation Committee of ISCT and EBMT (JACIE). Haematologica 2020;105(2):297–316.

Cell Therapy for B-Cell Lymphoma: CAR-T and Others

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The adoptive transfer of cancer-fighting immune cells is a rapidly developing immunotherapy in treating various lymphomas. Chimeric antigen receptor (CAR) T-cell therapies have demonstrated unprecedented efficacy in treating relapsed and refractory B-cell lymphoma, with few FDA-approved products now commercially available. Notably, the 5-year follow-up showed a significant portion of patients remained progress-free, suggesting a possible cure of the disease. Nevertheless, the cost and toxicity of CAR-T therapy remain of great concern. Moreover, previous chemo- and radiotherapy may negatively impact the optimal production of CAR-T. Natural killer (NK) cells, unlike T cells, mediate cytolysis independent of HLA class I expression. NK cells can also be collected, and ex vivo expanded from a killer cell immunoglobulin-like receptors (KIR)-mismatched allogeneic donor. These cells have more potent anti-tumor efficacy without causing graft-versus-host disease (GVHD). Cytokine-induced killer (CIK) cells are a heterogeneous group of CD8+ T cells. CIK can be easily and efficiently expanded from peripheral blood. Similar to NK cells, CIK lyse the cancer cells in an HLA-independent manner. CIK can also be obtained from healthy, allogeneic donors without significant risk of GVHD. Using the antibody-cell-conjugate (ACC) technique to introduce Rituximab or Obinutuzumab onto the cell surface of CIK may further improve the recognition and cytotoxicity against CD20-positive B cell lymphoma. More and more data support cell therapy would play a crucial role in managing B cell lymphoma. However, accessibility and the financial issue will be the major hurdle for wide application. In addition, cell therapy was used as monotherapy in the relapsed/refractory setting in the vast majority of the studies. Incorporating cell therapy into earlier stages of disease treatment and combining cell therapy with other effective anti-lymphoma agents is expected and should be explored in the future.

Anti-leukemic Therapies after Allogeneic Blood and Marrow Transplantation in Adult Patients with AML

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Despite the use of intensive chemotherapies followed by blood and marrow transplantation (BMT) at a timely adopted manner in many decades of trials, so many patients were yet finally relapsed or diagnosed as refractory disease. Together, the issues of most recent findings of the microenvironmental niches for keeping the immortal malignant leukemic stem cells (LSCs) as well as the measurable residual diseases (MRDs) even after the allogeneic BMT are still on many more challenges. When it comes to the concept of maintenance after BMT, nowadays researchers are focusing on the selective immunomodulation using niche-refreshing trials combined with specific or non-specific conventional immunotherapies, as a major promising part of treatment against chemoresistant acute leukemias.

The conventional concept of adoptive immunotherapy using stratified vaccine trials, antigen-specific CTLs, CAR-T/NK, bispecific killer-cell engager T, and gene transferred mesenchymal stromal cells (MSCs) as well as the combined use of various immunomodulating novel agents or well-designed sequential approaches that have been introduced nowadays should be applied to many high-risk patients with acute leukemias that are desperately in need to prevent relapse or for the relapse prophylaxis.

I like to introduce the background of anti-leukemic cytotoxic T-cells (CTLs) therapy for high-risk AML through some experiences in my institution. Based on the previous reports of sustained clinical remission accompanied by T cell responses after infusing the in vitro cultured *Wilms tumor gene 1* (*WT1)*-specific T cells which were expanded in vivo to elicit long-lasting anti-leukemic immunity in allo-BMT setting, it should be still one of good ways of applying the personalized care in patients with high-risk AML.

Timing of allogeneic transplantation: the knowns and the known unknowns

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Patients with acute myeloid leukemia (AML) in complete remission (CR) and myelodysplastic syndrome (MDS), generally need allogeneic hematopoietic cell transplantation (HCT) for cure. The comparison of when or whether to transplant requires consideration of alternatives and a lost curative opportunity if non-transplant therapies are tried first. Patients' age and comorbidities plus complications acquired during induction can influence risks of non-relapse mortality (NRM). If transplantation is deferred beyond CR1, even those with favorable molecular and cytogenetic risk still can face early progression. Intermediate and high-risk features favor early transplantation. Even for AML in CR1, a greater number of cycles to achieve CR1 and measurable residual disease (MRD) pre-transplant compromise the likelihood of success. MDS patients have no alternative curative option but progression beyond early stage MDS greatly limits HCT success. A promptly available suitable allogeneic donor is essential. While HLA matched siblings and unrelated donors (URD) generally yield similar outcomes, URD delayed availability compromises those needing an urgent transplant. Umbilical cord blood (UCB) and haplo-identical related donor grafts, the latter revolutionized by post-HCT cyclophosphamide, are available quickly, though the completeness of immune constitution, the hazards of infection and risks of relapse remain uncertain. UCB is limited by greater risks of graft failure and NRM. All these factors need to be discussed with patients to assess their willingness to assume risks in hopes for better outcomes. Older patients may be unwilling to assume greater risks and accept the unknowns that accompany the decision for transplantation during CR1. Careful patient counseling and intensive supportive care is essential to minimize the hazards and maximize the benefit of early allografting.

Infection and malignancy targeted cell therapy in combination with purified stem cells as a paradigm for allogeneic stem cell transplantation

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Despite changes in transplant techniques and drug therapy of complications, the paradigm of allogeneic stem cell transplant has not substantially changed for over 50 years. The contradictory tensions between graft versus host disease, relapse and infection remain unbroken and attempts to resolve any one of these complications are frequently frustrated by induction of the other(s) or amplification of the initial problem. We propose a strategy that involves isolation of donor CD34+ blood stem cells that are then used for transplantation in conjunction with ex vivo expanded T-cells from the stem cell donor targeted to common infectious and malignant antigens. We propose that less common infectious antigens can be addressed using partially HLA matched third party T-cells from cryopreserved cell banks. In work so far, we have tested donor-derived CMV and EBV specific T-cells and shown that they reduce the incidence and severity of post-transplant viral infection. More recently we have combined these with donor-derived cells targeting myeloid antigens WT-1 and PRAME and used donor derived CAR19 T-cells to treat patients with relapsed lymphoid leukaemia after allogeneic transplant. In a pilot study, two patients with acute lymphoblastic leukaemia undergoing transplant received CD34+ stem cells combined with donor T-cells targeting CMV, EBV and Aspergillus and locally manufactured donor derived CAR19 T-cells. Both patients are alive with no evidence GVHD or relapse over 1 year post-transplant. Graft engineering to improve cell specificity carries the promise of unlinking the major complications of allogeneic stem cell transplant to improve outcomes.

Common drug interaction and management in BMT patients

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Now a day, hematopoietic stem cell transplantation (HSCT) is a standard treatment to curative in relapsed and refractory hematologic malignancy patients. Patients are treated with complicated medications included chemotherapies, immunosuppressives, and antibiotics. In addition, some HSCT patients are receiving polypharmacy. So, those of medication are used together drug-drug interaction can happen. A drug-drug interaction (DDI) could cause a therapeutic problem as soon as the impact on a clinical parameter reaches the level of clinical relevance. DDI can be explicated by pharmacokinetic and pharmacodynamic drug interaction. The most reason that we can be found in pharmacokinetic drug interaction that is related to absorption, distribution, metabolism, and elimination which is come from affecting oral drug absorption, renal function, liver function, cytochrome P450(CYP) status and low serum albumin. Those can alter the serum drugs level which can bring about increase drug toxicity or drug less effective. However, some drug-drug interactions may be designated in theory, all these interactions are not necessarily clinically significant.

In this session describe and discuss about the potential mechanism and management of common drug-drug interactions. The example of drug-drug interactions is chemotherapy, busulfan, and cyclophosphamide, with supportive care medications, NK-1 receptor antagonists, 5HT3 receptor antagonists and antiepileptic drugs, immunosuppressive drugs, cyclosporine, tacrolimus, sirolimus, and mycophenolic acid with azoles, fluconazole, itraconazole, voriconazole and posaconazole. In conclusion of this session, focus primarily on the clinically relevant pharmacokinetic drug interactions that occur in transplantation regimens and offer recommendations for preventing and/or managing those adverse events.

Managing Patients receiving CAR-T Cell Therapy from Pharmacist Perspective: Our Singapore Experience

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Patients receiving chimeric antigen receptor (CAR) T-cell therapy are at an increased risk for opportunistic infections due to the cumulative effect of prior treatments. Infections are one of the most common side effect of CAR T-cell therapy as patients have prolonged neutropenia. There is no clear recommendation on antimicrobial prophylaxis in CAR-T patients and most studies done adopted standard practices extrapolated from the allogeneic stem cell transplantation. The current practices for antimicrobial prophylaxis at Singapore General Hospital were collated from international guidelines and best practices around the world. For anti-bacterial prophylaxis, our preferred fluoroquinolone is oral ciprofloxacin. In terms of anti-viral prophylaxis against herpes virus, we proposed oral acyclovir. In view of risk for cytomegalovirus (CMV) viremia beyond 30 days of CAR T-cell therapy, CMV monitoring may be considered every 1-2 weeks. Anti-pneumocystis prophylaxis with co-trimoxazole or pentamidine is mandatory for all patients receiving CAR T-cell therapy in view of fludarabine-based lymphodepleting conditioning used. As for anti-fungal prophylaxis, the approach is less straightforward and dependent on patients' risk factors. Our primary antifungal prophylaxis is fluconazole. At-risk patients should receive Posaconazole. Patients with pre-existing fungal infections, the choice of secondary prophylaxis should be made in consultation with an Infectious Disease physician. Routine use of granulocyte colony stimulating factors (GCSF) is not practiced at SGH. However, GCSF may be considered after day 5 of CAR-T, if clinically indicated and there is no cytokine release syndrome and IEC-associated neurotoxicity syndrome. Intravenous immunoglobulin replacement at 0.25 g/kg is recommended when serum IgG <4 g/L.

Transplant coordination in the CoVID19 era: changes and challenges, an Australian experience

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Australian international borders closed in March 2020 in response to the global CoVID-19 pandemic. This placed unprecedented stress on the health care system including hospitals performing allogeneic and autologous hematopoietic cell transplant (HCT). St Vincent's Hospital Sydney performs approximately 50 allogeneic transplants annually, more than half these have unrelated donors from overseas registries in Europe, UK and USA. Traditionally cells had been collected on day -2 and been transported using couriers on commercial flights, arriving on day 0 for infusion to the patient. Our medical, scientific and nursing staff quickly adopted new practices to minimise potential harm caused by delays resulting from the pandemic. Securing the national or international donor cell product before patient conditioning by cryopreserving at collection centre and sending to Sydney via air freight, often after quarantine in the country of origin, was one of the biggest changes in our practice since 2020. This risks the donor cells not being used such as in the event of relapse. Other challenges include change from in-person meetings to a virtual format of ward rounds and education sessions to minimise staff attending the ward in person, visiting hours were severely restricted, causing distress for many patients and families. Our outpatient clinic moved to minimise face to face visits with a shift to telehealth wherever possible. Related donors residing overseas had to apply for exemption to travel to Australia and were subject to strict 14 day hotel quarantine before the donation process. Case studies will be presented to illustrate the impact of these sudden changes during this extraordinary global crisis on our patients, donors, staff and our community.

No conflict of interest to disclose.

HOME CARE & FOLLOW UP AFTER HSCT

Amalorpavamari Lucas

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Hematopoietic Stem Cell Transplantation (HSCT) refers to a complex procedure which involves replacement of diseased marrow with healthy donor hematopoietic stem cells. HSCT is the main treatment for many Hematological malignancies and genetic disorders. Patients after HSCT are at risk of developing complications due to the conditioning regimen and radiation. Nurses need to prepare the patients for home care management. The homecare instructions should be focused on the following aspects: infection control, nutrition and fluid management, personal care, CVAD care, activity and exercise, GvHD management, drugs, supportive therapy and where & when to report to the physician. Post allogeneic HSCT patients must adhere to the follow-up protocol of the institution. The purposes are to monitor delayed complications, to monitor chimerism & to adjust drug dose. The components of follow-up care include physical assessment, investigations, immunosuppressants dose adjustment, chimerism and CMV PCR monitoring, assessment of delayed complications and immunization. Allogeneic HSCT poses great risk for the patients and has a significant impact in their quality of life. There is a tremendous role of Haematology Nurses in every phase of transplant process. This demands nurses to be highly up-to-date in their knowledge and clinical competence to ensure quality and safe patient care.

Transplantation and pharmacokinetic monitoring

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Allogeneic hematopoietic cell transplantation (HCT) is a potentially life-saving procedure by transplanting donor-derived hematopoietic stem cells and lymphocytes into to a patient. The technique is also referred to as stem cell transplantation, however this is not fully correct due to co-infusion of lymphocytes and other hematopoietic cells. Indications for HCT include malignant (leukemia, lymphoma) and non-malignant disorders (primary immune deficiencies, bone marrow failure, inborn errors of metabolism and hemoglobinopathies). During this procedure, the diseased bone marrow and cellular immune system is replaced by a healthy, donor-derived hematopoietic system.

The treatment plan for HCT depends on the disease, age, comorbidities, previous treatments, stem cell source and local protocols, and can therefore vary considerably between patients. Still, the main components for any HCT are the same, and are depicted in figure 1. In my presentation I will mainly focus on the impact of pharmaco-kinetics (drug exposure) of agents given in the *conditioning regimen* (and individualized dosing using population-PK models and therapeutic drug monitoring) and on *immune reconstitution* and the subsequent impact on the transplantation-related outcomes, such as transplantation-related mortality, graft-versus-host disease and survival. The major limitations of HCT include 1) transplant-related mortality, 2) relapse of disease, and 3) late effects.

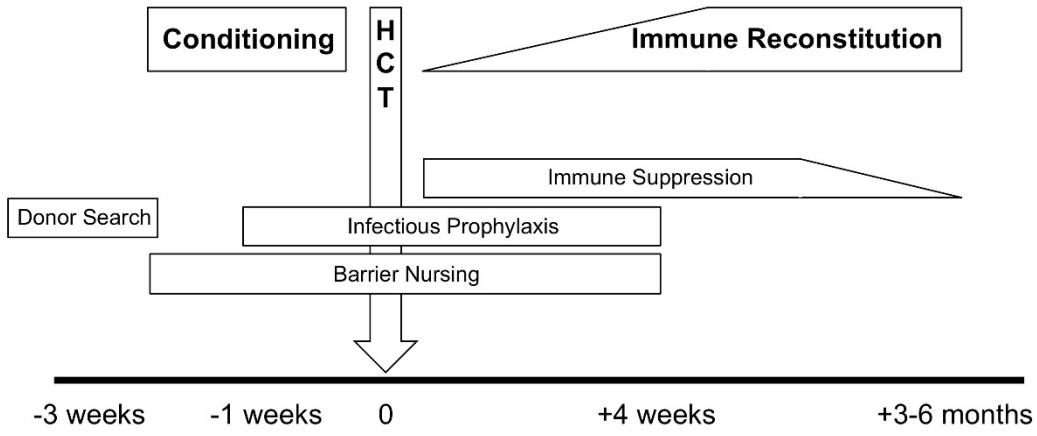

Figure 1: Overview of treatment plan for HCT

The *conditioning* phase starts approximately 7-10days before infusion of the stem cells, however some centers start conditioning earlier. The main goal of the conditioning is to deplete the bone marrow and suppress the host immune system. Additionally, in case of malignancy, the conditioning regimen depletes any residual leukemic cells. Bone marrow depletion, or myeloablation, is mostly performed using chemotherapy, while some patients receive

chemotherapy combined with total body irradiation (TBI). Chemotherapy-based conditionings mostly consist of an alkylating agent (busulfan, melphalan, treosulfan) combined with a second cytostatic drug (fludarabine, cyclophosphamide). The alkylators mainly give myeloablation, while fludarabine and cyclophosphamide are used for immunosuppression and immune-ablation. Clofarabine, a purine antinucleotide, can be added to the conditioning regimen for malignant indications. In TBI-containing regimens, TBI is used for myeloablation as well as immunosuppression, and is combined with one or more cytostatic drug. In recent years, non-myeloablative regimens or reduced intensity conditioning (RIC) has been increasingly used for older patients (>60 years) and those in poor clinical condition.

Besides differences in choice of drugs, the actual drug exposure varies due to variability in pharmacokinetics (PK) and pharmacodynamics (PD) between patients. By using individualized dosing regimens, this variability in PK and PD is accounted for, resulting in more patients reaching optimal drug exposure and thereby drug effects. An individualized dosing regimen is available and being used in clinical care for busulfan (Bartelink et al, 2016);

The optimal therapeutic window of busulfan exposure has been established in multiple reports[5,8]. This optimal exposure appears to be independent on cell source, match grade, indication and concomitant conditioning agents. Although the optimal exposure was similar when receiving 1 (Busulfan as single alkylator), 2 (Busulfan combined with cyclophosphamide; Cy) or 3 alkylators (Busulfan, Cy and Melphalan), patients receiving only busulfan combined with fludarabine had lowest toxicity and superior overall survival chances (due to lower toxicity: e.g. veno-occlusive disease [VOD], graft versus host disease [GvHD] and idiopatic pneumonia syndrome [IPS]). The optimal cumulative target exposure for Bu $AUC_{0\text{-}4\ days}$ was found to be 90 mg*h/L (range 80 – 100 mg*h/L, over 4 days), for all cell sources, including cord blood (Long-Boyle, 2011; Bartelink et al; 2016). Also, for fludarabine and optimal exposure was found in pediatrics and adults. Over exposure (cumulative >25mg*h/L) was found to be a predictor for transplantation-related mortality due to hampered immune reconstruction (see also below). Currently, a randomized controlled clinical trial is open in the Netherlands; standard BSA based versus individualized based dosing (where GFR and weight, the found predictors for clearance, are the variables for dosing) (Langenhorst et al; 2019). Additionally, exposure of ATG (Thymoglobuline), a lymphodepleting poly-clonal antibody, before and after HCT has shown to have impact on the outcomes. Individualized dosing regimen for ATG seems therefore crucial to influence the outcomes; currently clinical studies for individualized ATG are recruiting. One of these trials recently presented their results and clearly showed an significant impact on probability to attain early CD4+ immune reconstitution (of 50CD+ at 2 consecutive timepoints within 100days), which was found to be a very sensitive ‘marker’ for predicting transplantation related mortality in different transplantation platforms (Lindemans et al; 2014; Admiraal et al; 2017 2x, 2015, De Koning et al; 2017).

In addition to HCT, also in autologous-gene-transduced transplantation (GT; for monogenetic diseases such as sickle cell disease, lysosomal storage diseases) and in CART cell therapy the exposures of agents used in the conditioning regimen was found to be a predictor for outcomes. Sessa et showed in the lentiviral GT trial in early-onset metachromatic leukodystrophy that patients with sub-ablative exposure of busulfan had lower engraftment of gene-transduced cells, resulting in lower (not supra-normal; which was the goal of this intervention) enzyme levels[12]. Patients with ablative ($AUC_{0\text{-}4\ days}$ of 80-100 mg*h/L) busulfan

exposure achieved supra-normal enzyme levels (5-10 times normal) (Sessa et al; 2016). Also, in other autologous gene-transduced HCTs, aka GT, (e.g. Wiskott-Aldrich, Fabry disease, Beta-thalassemia) sufficient ablation seems important for optimal effect. For most GT Bu90 is considered the standard. In CART cell therapy Fludarabine exposure seems important. Patient with lower exposure (<14mg*h/L cumulative) were found to have a higher probability on relapse.

HCT provides a final and potentially curative treatment option for several malignant and benign disorders. However, there is a need for improved survival chances after HCT, which may be accomplished by improving disease control and reducing the toxicity of the procedure. Pharmacotherapy plays a major role in the conditioning phase; highly variable pharmacokinetics resulting in highly variable outcomes. There is a stringent need for an evidence-based, individualized dosing regimen agents used in HCT.

References

1 Lindemans CA, Chiesa R, Amrolia PJ, *et al.* Impact of thymoglobulin prior to pediatric unrelated umbilical cord blood transplantation on immune reconstitution and clinical outcome. *Blood* 2014; **123**: 126–32.

2 Langenhorst JB, van Kesteren C, van Maarseveen EM, *et al.* Fludarabine exposure in the conditioning prior to allogeneic hematopoietic cell transplantation predicts outcomes. *Blood Adv* 2019.

3 Long-Boyle JR, Green KG, Brunstein CG, *et al.* High fludarabine exposure and relationship with treatment-related mortality after nonmyeloablative hematopoietic cell transplantation. *Bone Marrow Transplant* 2011; **46**: 20–6.

4 Storek J, Mohty M, Boelens JJ. Rabbit Anti–T Cell Globulin in Allogeneic Hematopoietic Cell Transplantation. *Biol Blood Marrow Transplant* 2015; **21**: 959–70.

5 Admiraal R, de Koning C, Lindemans CA, *et al.* Viral Reactivations and Associated Outcomes in Context of Immune Reconstitution after Pediatric Hematopoietic Cell Transplantation. *J Allergy Clin Immunol* 2017; **ePub**.

6 Admiraal R, van Kesteren C, Jol-van Der Zijde CM, *et al.* Association between anti-thymocyte globulin exposure and CD4+ immune reconstitution in paediatric haematopoietic cell transplantation: a multicentre , retrospective pharmacodynamic cohort analysis. *Lancet Haematol* 2015; **2**: e194–e203.

7 de Koning C, Admiraal R, Nierkens S, Boelens JJ. Immune reconstitution and outcomes after conditioning with anti-thymocyte-globulin in unrelated cord blood transplantation; the good, the bad, and the ugly. *Stem cell Investig* 2017; **4**: 38.

8 Bartelink IH, Lalmohamed A, van Reij EML, *et al.* Association of busulfan exposure with survival and toxicity after haemopoietic cell transplantation in children and young adults: a multicentre, retrospective cohort analysis. *Lancet Haematol* 2016; **3**: e526–36.

9 Admiraal R, Nierkens S, de Witte MA, *et al.* Association between anti-thymocyte globulin exposure and survival outcomes in adult unrelated haemopoietic cell transplantation: a multicentre, retrospective, pharmacodynamic cohort analysis. *Lancet Haematol* 2017. doi:10.1016/S2352-3026(15)00045-9.

10 Sessa M, Lorioli L, Fumagalli F, *et al.* Lentiviral haemopoietic stem-cell gene therapy in early-onset metachromatic leukodystrophy: an ad-hoc analysis of a non-randomised, open-label, phase 1/2 trial. *Lancet* 2016; **388**: 476–87.

Allogeneic SCT for elderly with hematological malignancies

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Recently, allo-SCT has increasingly been used in elderly population. Development of less toxic pre-transplant conditioning and diversified donor types including HLA-mismatched relatives and cord blood as grafts available are the main reasons. However,
the outcomes reported so far have not been satisfactory. The OS of the elderly is reported to be 30-40% [1-6], and higher incidence of non-relapse mortality than in younger patients is one of the issues needs to be resolved urgently.

Elderly patients were reported to be more vulnerable to immune-related complications than younger patients [7,8]. In cord blood transplantation (CBT), which almost half of the elderly patients (≥65 years) choose as a graft in Japan, overall survival of those who had preengraftment immune reactions (PIR), allo-immune reactions unique to CBT develop during neutropenic period before engraftment, was inferior to those who did not in elderly patients [8]. Intensified GVHD prophylaxis reduced the incidence of severe form of PIR, and resulted in better survival for those with RIC conditioning [9]. Regarding the treatment of steroid-refractory GVHD, mesenchymal stem cells that recently introduced showed better response rate compared to the other treatment [10,11]. In western countries, ruxolitinib showed better response rate than the other best available treatments [11].

Another issue is the higher relapse incidence compared to the younger population. The malignant cells in the elderly tend to harbor cell intrinsic characteristics such as molecular or cytogenetic abnormalities with poor response rate to chemotherapy [12]. Intensified conditioning using intravenous busulfan along with fludarabine has increasingly been used and has been successful reducing relapse incidence in elderly patients [13]. Toxicities associated with the regimen, however, is still higher than in younger patients. Combining various drugs or TBI with reduced dose is the next approach currently investigated in our institute [14]. In addition, newly introduced drugs with novel mechanisms of action such as molecularly-targeted or immune-mediated, distinct from the ordinary chemotherapeutic drugs, have been successful in controlling diseases before or after transplant without increased toxicity [15-17]. These newer drugs may help solving the dilemma, allogeneic transplantation has been facing for a long time, that attempts to reduce NRM increase the incidence of relapse and vice versa.

References

1. Uchida N, et al. Int J Hematol. 2020;112:510.
2. Isobe M, et al. Ann Hematol. 2021;100:1849
3. Hsu J, et al Biol Blood Marrow Transplant 2020;26:789.
4. Muffly L, et al. Blood 2017;130:1156.
5. Santoro N, et al. J Hematol Oncol. 2018;11:55.
6. Aoki J, et al. Am J Hematol. 2016;91:302.
7. Nakane T, et al. Leuk Lymphoma. 2015;56:2392.
8. Uchida N, et al. Biol Blood Marrow Transplant. 200;14:583.
9. Uchida N, et al. Transplantation. 2011;92:366.
10. Muroi K, et al. Int J Hematol.. 2016;103:243.
11. Zeiser R, et al. NEJM 2020;382:1800.

12. Appelbaum FR, et al. Blood. 2006;107:3481.
13. Yamamoto H, et al. Biol Blood Marrow Transplant. 2015;22:1844.
14. Takagi S, et al. JSHCT 2020;O4-5.
15. Stone RM, et al. N Engl J Med;377:454.
16. Burchert A, et al. J Clin Oncol. 2020;38:2993.
17. Xuan L, et al. Lancet Oncol. 2020;21:1201.

Transplantation in Special Population

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Immunoglobulin light chain (AL) amyloidosis is a systemic disease in which instable monoclonal light chains or light chain fragments produced by an abnormal plasma cell clone are deposited as amyloid fibrils and lead to organ dysfunction. Kidney the most commonly effected organ with clinically relevant involvement seen in up to 70% of cases, and approximately 40% of those progress to dialysis dependence (1, 2) causing impairment of quality of life and shortened survival.

Cytotoxic therapy directed against the abnormal plasma cell clone with the goal of suppressing the amyloidogenic free light chains is the mainstay of treatment of AL amyloidosis. Autologous stem cell transplant (ASCT), a potent plasma cell directed therapy has been an important part of AL treatment for many years despite lack of randomized trial data supporting its use. In fact, a small randomized trial reported ASCT to be inferior to melphalan and dexamethasone combination.(3) However, in that study, of the 50 patients randomized to ASCT arm only 37 actually underwent transplant and 9 whom died before 100 days - an unacceptably high100-day transplant related mortality (TRM) of 24%. Moreover, in a 6-month landmark analysis similar survival was noted in the ASCT and melphalan-dexamethasone arms suggesting that the survival disadvantage seen in the ASCT group can be attributed to very high TRM. In contrast, several recent studies have reported high complete response rates, including approximately 40% patients achieving complete hematologic remission, extended duration of response, improvement in organ function, improved survival, and low transplant related mortality rate of 1-4% in high volume centers. (4, 5) These studies also highlight the importance of careful patient selection to ensure optimal transplant outcomes. In recent years, many highly effective therapies (immunomodulatory drugs, proteasome inhibitors, monoclonal antibodies) that were developed for treatment of multiple myeloma have been successfully adapted for use in AL amyloidosis because of their superior efficacy(6) but ASCT can further enhance response and remains an integral part of overall treatment strategy for carefully selected patients.(7)

With the improvement in treatment the survival of patients with AL amyloidosis has remarkably improved in the last 2 decades. Consequently, as more patients survive with amyloid the burden of chronic kidney disease and dialysis dependence in this population can be expected to increase. Dialysis dependence in AL amyloid patients is associated with poor quality of life and worse life expectancy than typically seen in individuals with dialysis dependence from other causes.(8) The role of kidney transplant is AL amyloidosis has been controversial. Generally, AL amyloid patients with ESRD were not considered suitable candidates because of concern for recurrence of amyloid in the grafted kidney, disease progression in other organs, and poor outcomes noted in earlier studies.(9) Modern AL treatments are associated with higher rates of deep responses - complete remission or very good partial remission - that are often durable. Extended duration of disease control can be achieved in a substantial proportion of patients with AL amyloidosis with modern therapies, particularly in patients who are able to undergo ASCT making transplantation a more realistic option in AL amyloidosis patients with ESRD.

Contemporaneous studies have reported very encouraging kidney transplant outcomes in selected AL patients. A retrospective study from a large US center reported median survival from kidney transplant of 10.5 years in a cohort of 49 patients transplanted between 1987 and 2017.(10) The 1-, 3- and 5-year graft survival was 94%, 89% and 81%, respectively. Depth of hematologic response prior to kidney transplant was strongly predictive of transplant outcome; patients with CR or VGPR had median graft survival of more than 10 years. Thirty-three of the 49 patients (67%) had ASCT prior to kidney transplant. These outcomes are comparable to outcomes reported in non-amyloidosis patients.(11) Another retrospective analysis of kidney transplants in 51 AL amyloidosis patients from the UK found patient and allograft survival rates comparable to those of kidney transplant for diabetes nephropathy. This study also highlighted the importance of pre-transplant hematologic response on outcomes.(12)

These studies, although retrospective in nature, provide reason for optimism that combination of effective anti-amyloidosis therapies, including ASCT, to achieve deep hematologic responses followed by kidney transplant can be a viable strategy for selected patients with renal failure secondary to AL amyloidosis. Many questions such as the optimal timing of ASCT (in some situations ASCT after the kidney transplant may be considered), criteria for patient selection, any role of maintenance therapy for amyloidosis, and optimal post-transplant immune modulation will need to be investigated in future prospective studies.

References

1. Gertz MA, Lacy MQ, Dispenzieri A. Immunoglobulin light chain amyloidosis and the kidney. Kidney Int. 2002;61(1):1-9.
2. Gertz MA, Leung N, Lacy MQ, Dispenzieri A, Zeldenrust SR, Hayman SR, et al. Clinical outcome of immunoglobulin light chain amyloidosis affecting the kidney. Nephrol Dial Transplant. 2009;24(10):3132-7.
3. Jaccard A, Moreau P, Leblond V, Leleu X, Benboubker L, Hermine O, et al. High-dose melphalan versus melphalan plus dexamethasone for AL amyloidosis. N Engl J Med. 2007;357(11):1083-93.
4. Cibeira MT, Sanchorawala V, Seldin DC, Quillen K, Berk JL, Dember LM, et al. Outcome of AL amyloidosis after high-dose melphalan and autologous stem cell transplantation: long-term results in a series of 421 patients. Blood. 2011;118(16):4346-52.
5. Tsai SB, Seldin DC, Quillen K, Berk JL, Ruberg FL, Meier-Ewert H, et al. High-dose melphalan and stem cell transplantation for patients with AL amyloidosis: trends in treatment-related mortality over the past 17 years at a single referral center. Blood. 2012;120(22):4445-6.
6. Palladini G, Kastritis E, Maurer MS, Zonder J, Minnema MC, Wechalekar AD, et al. Daratumumab plus CyBorD for patients with newly diagnosed AL amyloidosis: safety run-in results of ANDROMEDA. Blood. 2020;136(1):71-80.
7. Muchtar E, Dispenzieri A, Gertz MA, Kumar SK, Buadi FK, Leung N, et al. Treatment of AL Amyloidosis: Mayo Stratification of Myeloma and Risk-Adapted Therapy (mSMART) Consensus Statement 2020 Update. Mayo Clin Proc. 2021;96(6):1546-77.
8. Gertz MA, Kyle RA, O'Fallon WM. Dialysis support of patients with primary systemic amyloidosis. A study of 211 patients. Arch Intern Med. 1992;152(11):2245-50.
9. Pasternack A, Ahonen J, Kuhlback B. Renal transplantation in 45 patients with amyloidosis. Transplantation. 1986;42(6):598-601.
10. Angel-Korman A, Havasi A. Kidney Transplantation in Systemic Amyloidosis. Transplantation. 2020;104(10):2035-47.
11. Wang JH, Skeans MA, Israni AK. Current Status of Kidney Transplant Outcomes: Dying to Survive. Adv Chronic Kidney Dis. 2016;23(5):281-6.

12. Law S, Cohen O, Lachmann HJ, Rezk T, Gilbertson JA, Rowczenio D, et al. Renal transplant outcomes in amyloidosis. Nephrol Dial Transplant. 2021;36(2):355-65.

ORAL PRESENTATION

Oral Presentation I

O-1-1

The outcome of hematopoietic stem cells transplantation (HSCT) in pediatric patients in COVID-19 pandemic: Impact of Delta Variate

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The current pandemic COVID-19 has a negative impact on chronically ill patients, and among them, hematopoietic stem cell transplantation (HSCT) candidates are not an exception.
The aim of this report is to investigate the influence of COVID-19 outbreask on the outcome of pediatric HSCT from the begning of the pandemic. We enrolled 160 patients (100 male and 60 female) with a median age of 8 years old who underwent HSCT between February 2019 and September 2021 at the Children's Medical Center , the largest pediatric HSCT center in Iran. The most common indications were ALL (n=42), AML (n=20), immune deficiency disorders (n=30), nonmalignant hematologic disorders (n=26), neuroblastoma (n=13), metabolic disorders (n=13), inherited bone marrow failure diseases (n=12) and lymphoma (n=4). Of all candidates, 143 underwent allogenic HSCT and 17 cases received autologous HSCs. Patients were divivded into two groups based on history of COVID-19 infection. 25 of 160 patients were infected with the COVID-19 virus, out of which 8 patients were infected during the pandemic of the non-delta variant and 17 patients in the delta variant of the virus. During the 20 month follow-up in COVID-19 pandemic, the overall survival (OS) were 77.8% and 52% in non-covid and covid group, respectively. 10 of the 12 deaths in the COVID-19 group were related to the delta variant. Our results also indicated that in the COVID-19 group 56% of patients was experienced GVHD out of which 78% of them were expired to had garde III and IV GVHD especially with gasterointestinal and liver involvment.
Overall, our results suggest that the incidence of COVID-19 infection as well as the mortality rate in pediatric patients who underwent HSCT were significantly raised, especially during the pandemic of the delta variant of the COVID-19 virus. Our findings also emphasize the importance of COVID-19 on the incidence of GVHD patients . However, further studies are required to more precisely investigate the effect of COVID-19 control during HSCT

Keywords: COVID-19, Hematopoietic stem cell transplantation, GVHD

O-1-2

Higher CD34+ cell dose and rapid natural killer cell recovery improve clinical outcome after transplantation in patients with myeloid malignancies

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Objective: The CD34+ cells dose in graft as well as early immune reconstitution are essential for the graft-vs-leukemia (GVL) effect to eliminate residual leukemia cells after allogeneic hematopoietic stem cell transplantation (allo-HSCT). This study is to elucidate the impact of CD34+ cell dose in graft on immune reconstitution and clinical outcomes after transplantation.
Methods: This study retrospectively analyzed 180 myeloid malignancies patients who received allo-HSCT between June 2012 and May 2021. We characterized NK cell in day30 after transplantation (NK30) in recipients with multicolor flowcytometry. The patients were divided into high- and low-CD34 groups or high- and low-NK30 group at the cutoff value of 4.5×106/kg and 150/ul, respectively . The cumulative incidence of relapse (CIR) was assessed using competing risks from the time of HSCT to death or relapse.
Results: Total 180 patients were enrolled, including 36 high-CD34 group and 144 low-CD34 group. The median follow-up time was 45.1 (1.9-107.5) month. The dose of CD34+ cells in graft correlated significantly with NK30 (Pearson R2=0.55, P<0.001), while there was no correlation to the transplanted dose of NK cells (Pearson R2=0.0002, P=0.84). Meanwhile, the high-CD34 group and high-NK30 group showed better 3-year relapse-free survival (RFS) (88.9±5.2% vs 65.1±4.0%, P=0.003; 85.9±4.3% vs 59.2±4.6%, P<0.001) and overall survival (OS) (88.9±5.2% vs 71.3±3.8%, P=0.005; 87.5±4.1% vs 70.5±4.3%, P=0.003) than did control group, without any increase in graft-versus-host disease (GVHD). Multivariate analysis revealed that the high-CD34 group and high-NK30 group was associated with higher RFS (hazard ratio [HR] 4.45, P=0.004; HR, 3.75, P<0.001), OS (HR, 3.27; P=0.024; HR, 2.91, P=0.006), and lower CIR (HR, 6.76, P=0.009; HR, 8.88, P<0.001).
Conclusion: Higher CD34+ cell dose in graft was beneficial to early NK reconstitution to support the enhanced GVL effect in patients with myeloid malignancies after transplantation.

Keywords: CD34+ cell, NK reconstitution, Relapse-free survival

O-1-3

The study of microRNAs in CD34+ HSPCs from AML patients with relapse after allogeneic transplantation

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Background: Relapse of the original disease is a major cause of death after allogeneic hematopoietic cell transplantation for acute myeloid leukemia (AML). It has been established that
AML is driven by a subpopulation of rare cells with intrinsically defined stem cell properties. However, the mechanism of post-transplantation relapses at the stem cell level is not clear.
Aim: To describe role of microRNA (miRNA) in CD34+ hematopoietic stem/progenitor cells (HSPCs) from AML patients with relapse after allogeneic transplantation.
Methods: Bone marrow samples from 5 AML patients who received allogeneic hematopoietic cell transplantation, of which 2 patients relapsed after transplantation (RE) and 3 patients remained complete remission (CR). CD34+ HSPCs were sorted by CD34+ microbeads and analyzed by RNA-sequencing on an Illumina Hiseq 2500 platform to characterized all of miRNAs. Gene expression levels between groups of CD34+ HSPCs were compared with degeR to find out differential miRNAs. We performed the GO (Gene Ontology) and KEGG (Kyoto Encyclopedia of Genes and Genomes) enrichment analysis for the target gene candidates of differential miRNAs to predict the potential function of relapsed specific microRNAs. A value of $P<0.05$ (2-sided) was considered to be statistically significant.
Results: Compared with CR patients, we found that 61 miRNA were upregulated and 31 are significantly downregulated. The analysis showed the total of 92 miRNAs to be signifcantly altered as shown in the heatmap in Figure 1. The target gene candidates of differential miRNAs were enriched in numerous Go biological process including: immune system process, cell cycle, apoptotic process, protein transport, intracellular signal transduction, protein phosphorylation and so on . The top 10 signifcantly ($P< 0.05$) affected pathways identified by GO analysis.
Conclusion: The present study proved that miRNAs play an important role in AML patients with relapse after transplantation. The result may improved understanding of the special molecular alterations in relapse after transplantation and may provide potential targets for future clinical applications.

Keywords: MiRNA, Relapse after transplantation, AML

O-1-4

Pre-transplant immune suppression prevents graft rejection in children with IFN-gamma receptor defect mendelian susceptibility to mycobacterial disease

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Introduction: The IL-2 and IFN-g pathway mediated immunity is essential for host defence against mycobacterium tuberculosis, atypical mycobacteria and intracellular organisms like salomonella. In mendelian susceptibility to mycobacterial disease(MSMD) due to IFN-g receptor defect, hematopoietic stem cell transplantation(HSCT) is a curative option. High levels of IFN-g in these children are hostile to stem cell proliferation and can mediate graft rejection.

Methods: We describe the outcome of children with MSMD due to IFN-gamma receptor(IFN-gamma R1 and R2) defect who underwent HSCT in our unit. In view of graft rejection in a child who received reduced intensity conditioning for a haploidentical HSCT and the high levels of IFN-gamma levels, pre-transplant immune suppression(PTIS) with 2 cycles of Fludarabine(40mg/m2 for 5 days) and dexamethasone(25mg/m2) followed by double volume plasma exchange, a myeloablative conditioning, peripheral blood stem cells and a high stem cell dose of 10 million cells per kg of recipient was performed. Anti-tubercular therapy was given prior to HSCT for disease control and post HSCT until immune reconstitution.

Results: Four children with IFN-gamma receptor defect underwent five HSCT in our unit between January 2016 and June 2021. The median age was 4 years(3 months -6 years). Three children underwent haploidentical stem cell transplantation and one child had a matched sibling (MSD) and family donor(MFD) HSCT. Graft rejection was seen in two children. One child received a myeloablative conditioning but only 5 million stem cells per kg from the MFD and ultimately died due to progressive disease. The other child received a reduced intensity conditioning and 3 million stem cells per kg from haploidentical donor. He underwent second haploidentical HSCT 5 years later and is having complete chimerism after graded whole blood DLI for mixed chimerism. Remaining three children who received myeloablative conditioning and 10million peripheral blood stem cells per kg are having a complete donor chimerism and free of any infection.

Conclusion: PTIS with fludarabine and dexamethasone followed by plasma exchange, use of myeloablative conditioning, providing high peripheral blood stem cell dose of atleast 10 million cells per kilogram are needed for durable engraftment and to prevent graft rejection in IFN-gamma defect MSMD

Keywords: MSMD, IFN gamma receptor defect, Pediatric HSCT

O-1-5

The outcome of peripheral blood HSCT with myeloablative conditioning regimen in MIOP patients

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Malignant infantile osteopetrosis (MIOP) is a rare hereditary disorder that presents in early childhood. The osteoclastic bone resorption activity dysfunction leads to generalized osteosclerosis, cranial neuropathies, hepatosplenomegaly, and bone marrow failure. Allogeneic hematopoietic stem cell transplantation (allo-HSCT) was known as a curative modality for most types of mutation in this disease. However, allo-HSCT is unlikely to improve manifestations in the case of neurological complications. Here, we report the result of HSCT in eleven MIOP patients (6 males, 5 females), who underwent transplants in Children's Medical Center, the largest pediatric Iranian hospital, from October 2016 to June 2021. The mean age of patients at transplant was nine months (range, 1.8 months to 8 years). Six patients were transplanted from HLA-identical siblings (54.54%), while matched other related donors (45.45%). All transplants utilized PBSC sources. The mean number of infused MNCs and CD34+ was 7.84 × 108 (range, 7 – 8.7) and 5.9 × 106 (range, 1.73 - 10.8) cell/kg, respectively. All patients received a myeloablative conditioning regimen consist of Busulfan and Cyclophosphamide. Furthermore, ATG was administered in another relative donor setting. Engraftment has occurred in 8 patients. Among them, 7 had complete chimerism and 1 had mixed chimerism. While three patients experienced graft rejection. The median time to PLT and Neutrophil engraftment was 12 and 10 days, respectively. With an average 17 months' follow-up (range, 1 - 54), eight patients were alive. The acute graft versus host disease (GvHD) developed in three cases (37.5%), while two patients (25%) developed limited chronic GvHD. The cause of death was the rejection (n = 2) and pneumonia aspiration (n = 1). Despite a small population of MIOP patients, our results showed that Allo-HSCT could stop disease progression. So, early diagnosis and subsequently timely HSCT would improve the patients′ quality of life. On the other hand, it should be known that despite the use of PBSC with the MAC regimen the rate of rejection is still higher than other diseases due to the nature of the disease, which causes stricture in the internal space of bone marrow.

Keywords: Malignant infantile Osteopetrosis, Hematopoietic Stem Cell Transplantation, Allogeneic

O-1-6

Tacrolimus offers an effective GVHD prophylaxis with a tolerable toxicity profile in pediatric hematopoietic stem cell transplantation

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Introduction: Calcineurin inhibitors continue to be the preferred prophylaxis against graft versus host disease (GVHD) in children undergoing hematopoietic stem cell transplantation. We aimed to analyze the impact of Tacrolimus in the prevention of graft versus host disease and its toxicity profile.
Methods: We included children under 18 years of age who underwent HSCT between January 2017 and December 2020 on Tacrolimus and excluded children who had a primary graft failure or switched over to other prophylaxis due to toxicity. Tacrolimus was initiated at a fixed dose of 0.25 mg BD in children less than one year of age and 0.5 mg BD beyond infancy, and we modified the dose based on trough levels. In addition, Tacrolimus was administered sublingually in children with mucositis.
Results: We analyzed data on a total of 214 children with a median age of 7 years (0.5-18). The indication for HSCT was a benign disorder in 165 children and for malignancy in 49. The source of stem cells was peripheral blood in 190, bone marrow in 22, and bone marrow and cord blood in 2 children. We observed hypertension leading to PRES in 10 children, but none required renal replacement therapy for AKI. The mortality rate was 19.1 % caused by sepsis (n=21) progressive disease(n=13) and grade 4 GVHD (n=5). The overall survival of the entire cohort was 84% at a median follow-up of 48 months.
Conclusion: Graded dose increments in oral Tacrolimus based on trough levels in combination with methotrexate provides optimal GVHD prophylaxis with 84% of acute GVHD being grade I/II with tolerable side effects. In addition, the sublingual route of administration during gut mucositis achieves adequate trough levels.

Keywords: Tacrolimus, GVHD Prophylaxis, Pediatric HSCT

Oral Presentation II

O-2-1

Safety, feasibility and healthcare cost differences between inpatient and outpatient mobilization chemotherapy for autologous hematopoietic stem cell transplantation in multiple myeloma: A single center experience

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Introduction: Mobilization chemotherapy for autologous hematopoietic stem cell transplantation in patients with multiple myeloma (MM), vinorelbine and high dose cyclophosphamide (VC), has been historically given in the inpatient (IP) setting. Due to rising bed occupancy rates and patients' preferences, our team has offered eligible patients an option to receive VC outpatient (OP) since 2018. Our study aims to audit the feasibility and safety of this initiative, and review potential healthcare-related cost savings.
Methods: Eligibility criteria for OP chemotherapy were developed by a multidisciplinary team based on age, functional status, medical comorbidities and social factors. The chemotherapy regimen was modified for an OP setting. A retrospective review was conducted for 35 MM patients (18 IP and 17 OP) who received VC for mobilization at our center from 2018 to 2019. Patient data were analyzed from the day of admission for VC (IP) or day 1 of VC (OP), to the day before admission for stem cell harvesting. Clinical charts were reviewed for unexpected complications. Costs incurred were calculated using the value-driven-outcome (VDO) informatics analysis of the hospital.
Results: There were no unexpected clinical complications or unplanned admissions in both groups. The median length of hospital stay for the IP cohort was 3 days, amounting to a saving of 51 hospital days over 2 years in the OP cohort. Median costs were 73% lower in the OP cohort. The difference was mainly due to certain costs not incurred in the OP setting. These included room charges and daily treatment fees (which accounted for an average of 65% of IP charges). Investigation costs were also 55% lower in the OP cohort, which could be attributed to more investigations being performed in the IP setting such as screening for methicillin-resistant Staphylococcus aureus and non-urgent radiographs performed after hours upon admission.
Conclusions: Our findings show that OP mobilization chemotherapy for MM is safe, feasible and associated with improved bed utilization and cost savings. This supports the ongoing paradigm shift of right-siting treatment services from the IP to OP setting so as to optimize utilization of healthcare resources.

Keywords: Autologous hematopoietic stem cell transplantation, Multiple myeloma, Health services research

O-2-2

Evaluating cost saving and value driven outcomes in implementing outpatient peripheral blood stem cell (PBSC) collection: A nurse-led quality improvement initiative

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Background: Shortage of hospital beds for elective admissions during the COVID-19 pandemic resulted in PBSC collection delays. To maintain the service, an initiative to transit the collection to the outpatient setting was undertaken by a team of nurses.
Objective: To evaluate the feasibility, safety, bed-day and cost savings from the transition of care to the outpatient setting.
Method: From April 2020 to March 2021, 60 consecutive patients were enrolled. Eligible criteria were determined. The current workflow was modified which included i) CD34 level (haematopoietic stem cells marker) checks to be carried out at the Apheresis Unit (AU), ii) patients to report to AU as the first case and iii) collection procedure to be led by the Advanced Practice Nurse (APN) and the AU nurses. Overnight admissions applied for patients needing Day 2 collection through a central venous catheter (CVC) or for monitoring post catheter removal. The outcomes monitored include harvest delays, clinical complications, unplanned admission rates and bed-days saved, and were compared with matched historical controls from 2019. Healthcare costs differences were obtained from Value Driven Outcome (VDO) analysis.
Result: With 60 patients undergoing PBSC collection at the outpatient setting, a total of 146 inpatient bed-days were saved. Thirty-nine patients consisting of lymphoma (N=14), Myeloma (N=7), and Healthy Donors (N=18) who had matched historical control were included for analysis. Twenty-seven patients had PBSC harvested via vein, and 12 via CVC. There were no harvest delays, no unexpected clinical complications, or unplanned admissions. Median costs were 16% lower in the outpatient cohort with highest cost savings in the outpatient vein cohort (53% reduction).
Conclusion: Outpatient PBSC collection is not only feasible but safe and effective. While the quality and cost effective care is maintained, the health spending is lowered without adverse effect to the patients.

Keywords: OUTPATIENT PERIPHERAL STEM CELL HARVESTING, OUTPATIENT PBSC

O-2-3

Eltrombopag improves platelet recovery after allogeneic hematopoietic stem cell transplantation

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Background: Eltrombopag has shown remarkable efficacy in treating thrombocytopenia after allogeneic hematopoietic stem cell transplantation (all-HSCT).
Methods: 37 patients administered Eltrombopag 50 mg/qd from +1 day after HSCT until platelet (PLT) >50x10^9/L or 1 month after HSCT. 45 patients in the same period applied thrombopoietin (TPO) after HSCT and served as a control group. The engraftment of PLT at 1 and 3 months after HSCT was analyzed in both groups.
Results: 37 patients enrolled in the study and administered Eltrombopag, 2 of whom withdrew from the study due to intolerable gastrointestinal reactions. A total of 35 patients eventually completed treatment with Eltrombopag. At 1 month after HSCT, all the patients in Eltrombopag group had PLT >25x10^9/L, while in TPO group 40 (88.9%) had PLT >25x10^9/L (P= .064). 31 (88.6%) patients in Eltrombopag group were with PLT >50 x10^9/L, while 26 (57.8%) patients in the TPO group were with PLT >50 x10^9/L (P= .003). In Eltrombopag group, 18 (51.4%) patients' PLT were over 100 x10^9/L, and in TPO group 19 (42.2%) patients were over 100 x10^9/L (P= .500). At 3 months after HSCT, in Eltrombopag group, 28 (90.3%) patients had PLT > 25x10^9/L, compared with 40 (88.9%) patients in TPO group (P>.999). While 24 (77.4%) patients in Eltrombopag group had PLT > 50 x10^9/L, and 32 (71.1%) patients in TPO group had PLT > 50 x10^9/L (P= .604). There were 16 (51.6%) patients in the Eltrombopag group with PLT > 100 x10^9/L whereas 22 (48.9%) patients in the TPO group (P>.999)
Conclusion: Eltrombopag can promote PLT recovery in the early phase post-transplantation.

Keywords: Eltrombopag, thrombopoietin, platelet recovery

O-2-4

The efficacy of ice flakes with syrup for cryotherapy to prevent chemotherapy-induced oral mucositis in patients undergoing autologous stem cell transplantation

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Background: Oral mucositis occurs in more than half of patients who undergo high-dose chemotherapy (HDT) conditioning regimen for autologous stem cell transplants (ASCT), causing significant physical and psychological impacts. Evidences show that oral cryotherapy by applying ice or ice chips during the administration of HDTdecreased the incidence of grade 3–4 oral mucositis.
Objectives: This study aimed to evaluate the effects of local cryotherapy using ice flakes with syrup (kakigori) on chemotherapy-induced oral mucositis in patients undergoing HDT conditioning regimen for ASCT.
Patients and Methods: Between January 2016 to August 2021, 109 patients who underwent HDT conditioning regimen for ASCT received oral cryotherapy by having ice flakes made from boiled water with syrup, started 15 minutes before conditioning regimens containing melphalan, busulfan, and methotrexate. Patients were instructed to continue taking ice flakes until 15 minutes after the completion of HDT by using the balloon sucking technique. Based on the world health organization (WHO) scales, the severity of mucositis was evaluated daily until the day of discharge. The data were analyzed using descriptive statistics.
Results: A total of 109 patients were included in this study. There were 50 myeloma patients receiving high dose melphalan(200mg/m2) and 59 lymphoma patients receiving BEAM regimen. Seven patients (6.4%) experienced oral mucositis. The majority of patients had grade 1 oral mucositis (n=5, 4.5%). One patient had grade 2 and one patient had grade 4 mucositis.
Conclusions: Oral cryotherapy by applying ice flakes with syrup wass an effective therapy for reducing the risk of oral mucositis. Sweetened oral cryotherapy may improve the patient's satisfaction and

Keywords: Mucositis, Autologous Stem Cell Transplantation, Cryotherapy

O-2-5

Outcomes of antiemetic protocol in patients undergoing hematopoietic stem cell transplantation based on real-world practice

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Background: Despite of multiple-day chemotherapy conditioning regimen using in hematopoietic stem cell transplantation (HSCT), a variety of antiemetic protocol was design to prevent chemotherapy induced nausea and vomiting (CINV) which is the most concern side effect during HSCT. The rationale of this was to explore the efficacy of antiemetic prophylaxis in patient undergoing HSCT on the real-world practice.
Method: A retrospective descriptive study was performed, collecting data from medical record during January 2017 to July 2021 in Maharaj Nakorn Chiang-Mai Hospital, Thailand. All patients received antiemetics prophylaxis before conditioning regimen were recruited. The primary endpoint is the rate of complete response (no rescue medication; CR) during chemotherapy (acute phase) and 48 hours after conditioning regimen (delay phase). Secondary endpoints are rate of no emesis and no severe nausea during chemotherapy and 48 hours after.
Result: 107 patients were enrolled in this study including autologous and allogenic HSCT. BEAM is most common conditioning regimen (43%; n=46). Antiemetic prophylaxis regimens with NK1-antagonist plus 5-HT3 antagonist and dexamethasone were administered at 83.2% (n=89). Regarding the NK1-antagonist, 71 patients received aprepitant and 18 patients for netupitant/palonosetron (NEPA). The CR rate at the overall phase was 28% (n=30). In the acute and delay phase, the CR rate was 71% (n=76) and 30.8% (n=33), respectively. There were no statistically significant differences in the group of antiemetic prophylaxis regimens. The results of secondary endpoints were shown in Figure 1.
Conclusion: Multiple antiemetic regimens were effective for CINV prophylaxis. To optimize emetic control in HSCT patients, multi-agent antiemetic regimens should be developed to prevent CINV in the delayed phase.

Keywords: antiemetic, Stem cell transplantation, real world practice

O-2-6

Autologous stem cell transplantation in an hiv-positive patient with multiple myeloma during the COVID-19 pandemic

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Introduction: There is an increased incidence of plasma cell disorders among HIV+ patients. The use of antiretroviral therapy (ART) has made hematopoietic autologous stem cell transplantation (HASCT) accessible to this population. However, the onset of the COVID-19 pandemic poses difficulties to conduct of HASCT due to immunosuppression and infection risk. No studies have been reported on this unique subset within the Asia-Pacific region during the pandemic. This study reports HASCT done in a tertiary institution in the Philippines on an HIV+ patient with multiple myeloma after asymptomatic COVID-19 infection.
Case Discussion: A 54-year old male, HIV+ since 2018, maintained on Lamivudine, Tenofovir, and Efavirenz presented with a gluteal mass. Biopsy revealed CD138+ plasmacytoma and bone marrow biopsy with plasma cell population at 2-5%. Serum immunotyping electrophoresis showed polyclonal gammopathy (gamma elevation at 25.9%). Serum free light chain (SFLC) assay revealed monoclonal gammopathy with kappa light chain involvement (ratio 1.78). Serum beta-2 microglobulin was elevated. Lytic lesions were documented in the parietal and frontal skull. Other examinations showed normocytic, normochromic anemia with normal serum calcium and creatinine.
He underwent five cycles of Bortezomib, Dexamethasone, and Cyclophosphamide with repeat SFLC showing stringent complete remission and subsequently maintained on Lenalidomide 10 mg daily. Pre-transplant work-up showed undetectable viral load with CD4 count at 333. Chemo-mobilization with Cyclophosphamide and G-CSF yielded adequate CD34+ stem cell collection. Prior to transplant, patient was diagnosed with asymptomatic COVID-19 thru nasopharyngeal RT-PCR testing and was placed on quarantine.
After documented negative COVID PCR, patient underwent high dose Melphalan conditioning (200 mg/m2) and proceeded with HASCT. Course was remarkable for neutropenic fever, oral mucositis requiring parenteral nutrition, and culture-negative diarrhea. Focus of infection was at central line (Enterobacter cloacae) with resolution after targeted antibiotics and central line removal. Neutrophil and platelet engraftment was noted on day +10. ART was continued throughout the transplantation with no adverse events. Patient was discharged on day +18.
Conclusion: HASCT in HIV+ patients with multiple myeloma is feasible among those with controlled HIV status. Appropriate precautions may limit foreseeable infection risk during COVID-19 pandemic. Long-term follow-up is needed to establish durability of treatment response.

Keywords: stem cell transplant, multiple myeloma, HIV

E-POSTER PRESENTATION

E-Poster Presentation

P-1

Educational intervention and implementation of central line insertion practices (CLIP) bundle

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Background: Patients with hematological malignancies are at increased risk of infection due to disease itself and chemotherapy. Infection remains a leading cause of death especially in hematopoietic stem cell transplantation patients. Among hospital-acquired infection, central line-associated blood stream infections (CLABSI) are one of the most serious infectious complication which associated with morbidity, mortality, and cost. Strategies for prevention of CLABSI include educational intervention to nursing team and adherence of Central Line Insertion Practices (CLIP) bundle.
Objectives: The propose of this study was to investigate the rate of CLABSI in Bone Marrow Transplantation (BMT) unit after providing educational intervention and routine practicing follow National Healthcare Safety Network (NHSN) CLIP Bundle.
Methods: This was a descriptive study conducted from January 2018 to December 2020 at BMT Unit, Maharaj Nakorn Chiang Mai Hospital, Thailand. Patient who had a central venous catheter inserted during admission period were included in the study. The educational interventions consist of knowledge sharing and reviewing NHSN CLIP bundle which guided by an infection control nurse. CLIP bundle adherence and implementation were assessed by using protocol checklist. CLABSI rate was recorded and calculated as the number of infections per 1000 central line-days.
Results: CLABSI occurred 5.05 per 1000 catheter-days in 2018. After the intervention and implementation of protocol, the CLABSI incidence rate in 2019 and 2020 dropped to 2.4 per 1000 catheter-days and 4.06 per 1000 catheter-days, respectively. The CLIP bundle adherence was 100%.
Conclusion: Educational intervention and implementation of CLIP bundle result in a decrease in CLABSI rate. However, routine regular educating and good adherence to the bundle is important to prevent and maintain low rate of CLABSI.

Keywords: CLABSI, CLIP bundle, NHSN

P-2

Patient profiles and outcomes among lymphoma patients who underwent autologous stem cell transplant in national kidney and transplant institute (NKTI): A single-center analysis

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Introduction: Autologous stem cell transplantation (ASCT) is the standard of care for relapsed or refractory lymphoma for over 50 years and is associated with better overall survival compared to those who received chemotherapy alone. In the Philippines, no study has been done among lymphoma patients who received ASCT and their corresponding outcomes. The National Kidney and Transplant Institute (NKTI) pioneered stem cell transplantation in the Philippines in 1990, however, transplantation in lymphoma started only in 2019. This is the first local study to review ASCT outcomes among lymphoma patients.
Methods: This is a retrospective review in a single-institution of adult lymphoma patients who underwent autologous stem cell transplant (ASCT) from February 2019 to December 2020 in NKTI.
Results: There were eight (8) adult lymphoma patients who received ASCT from February 2019 to December 2020. Most patients (7 of 8) were diagnosed with Hodgkin Lymphoma and all had relapsed/refractory disease with at least 2 lines of treatment received prior. High dose chemotherapy or the conditioning regimen given to all patients was BeEAM (bendamustine/ etoposide/ cytarabine/ melphalan). Neutrophil engraftment was observed after an average of 11.4 days and patients were discharged on an average 16.2 days after transplantation. One patient died during admission, one patient still has not yet been re-evaluated, one patient relapsed, and five patients remained in remission- the longest duration being 2 years.
Conclusion: Autologous stem cell transplant can be safely and successfully performed in this institution. Immediate outcomes are comparable to global data, however, longer-term follow up is recommended to further determine its durability in terms of disease control and survival in the local setting.

Keywords: lymphoma, autologous, transplant

P-3

Satisfaction of patient education and initial consultation by online communication application in patients receiving stem cell transplantation

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Background: The side effects of high-dose chemotherapy and stem cell transplantation remain a major concern for both patients and clinicians. Continuing patient education during admission and after hospital discharge is one of the effective processes that help prevent or lessen further complications. Online communication platform such as “LINE official” is commonly used in many countries such as Japan, Taiwan, and Thailand. We hypothesized that “LINE official” platform conveniently provided effective communication and prompt initial management dealing with post-transplant complications in those patients.
Objectives: This study aimed to investigate the satisfaction of stem cell transplant patients who received health education and initial consultation using “LINE official” application.
Methods: Study characterized as descriptive, undertaken from October 2019 to April 2021 at Stem cell Transplantation Unit, Chiang Mai University Hospital. All patients admitted for stem cell transplant during study period and had smart phone using “LINE official” application were included in this study. Health education materials including video and infographic for patient’s guidance after discharge from hospital would be provided via the platform. Patient could communicate with health care workers which available all the time. We collected data from questionnaire to assess patient’s satisfaction, patient’s basic knowledge, and health care worker’s satisfaction. The adverse events were recorded during study period.
Results: There were 51 patients who undergoing stem cell transplant during study period and 40 patients (78.4%) had “LINE official” application. Mean satisfaction score for patients was 92.6% (95% confidence interval (95% CI) 91.4 - 93.8) (Figure 1.). Patient’s knowledge score was 93.9% (95% CI 92.5-95.3). There was no patient who had major bleeding or falling during study period. Health care workers who used “LINE official” to provide patient’s information also satisfied with this application with the mean score of 83.8% (95% CI 82.9-84.7).
Conclusion: “LINE official” application was an effective platform in order to provide prompt communication, and on-going health education for patients receiving stem cell transplantation. There was good satisfaction from both patients and health care workers. The impact of this intervention on patient’s outcomes including survival rate and adverse events should be further investigated.

Keywords: Health education, Stem cell transplantation, LINE application

P-4

A prospective study on efficacy and safety of filgrastim (Leuco-Plus®) for prevention of chemotherapy induced neutropenia in patients with diffuse large B-cell lymphoma (DLBCL)

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Diffuse large B-cell lymphoma (DLBCL) is the most common hematologic malignancy. CHOP-based with/without rituximab regimen is the mainstay primary treatment for this disease, while prophylactic use of filgrastim is vital for those who have high risk factor(s) for chemotherapy-induced neutropenia (CIN).

This prospective study investigated the prophylactic effect of Leuco-Plus® on CIN and its safety among Thai patients with newly diagnosed DLBCL treated with CHOP ± Rituximab.

Eligible patients planned for 3-weekly CHOP ± Rituximab together with filgrastim primary prophylaxis were recruited. All patients received Leuco-Plus® within the first 2 cycles at dose of 5 mcg/kg/day, which could be initiated after 24-72 hours post chemotherapy. Its duration could be extended up to Day14 based on physician's discretion and hematology results those were performed on Day0, Day7, Day10, and Day14 of each cycle. Rate and duration of neutropenia/febrile neutropenia (FN), duration of Leuco-Plus treatment and adverse events (AEs) were assessed. The study ended on Day0 of cycle 3.

Twenty-one participants (median age, 62.8 years old) were enrolled; 57.1% was in stage 3 or 4 DLBCL and 42.9% had bone marrow involvement. R-CHOP was given in 15 (71.4%) and 18 (85.7%) patients for the 1st and 2nd cycle, respectively and most started Leuco-Plus® on Day4 of each cycle. Mean (±SD) durations of Leuco-Plus® treatment were 7.57 (1.76) and 6.67 (2.49) days for cycle 1 and 2, respectively. None of the patients experienced FN or delayed chemotherapy. Eight and four patients developed grade 3/4 neutropenia, mostly on Day10 of cycle 1 and 2, respectively. On Day14 of both cycles, they were all resolved except one patient (4.8%) with grade 3 neutropenia in cycle 1. Mean (±SD) durations of grade 3/4 neutropenia were 4.90 (2.02) and 5.67 (0.47) days for cycle 1 and 2, respectively. Mild bone pain related to Leuco-Plus® were reported in 4 patients. There was no serious AE related to Leuco-Plus®.

This study demonstrated efficacy and safety of Leuco-Plus® in primary prophylaxis of CIN among newly diagnosed DLBCL patients treated with CHOP ± Rituximab. Neither FN nor delay of chemotherapy was reported. No significant AE related to Leuco-Plus® was observed.

Keywords: chemotherapy-induced neutropenia, Diffuse large B-cell lymphoma, filgrastim primary prophylaxis

P-5

Anti-fungal and tuberculosis infections of a patient with hematologiccal malignancies: A case report and literature review

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Background: Patients with hematological malignancies also have tuberculosis, which is a high-risk group of other infections. We applied posaconazole and rifapentine to treat a patient with acute myeloid leukemia complicated by the probable invasive aspergillus and tuberculosis infection.
Case presentation: A 68-year-old male patient with acute myeloid leukemia was diagnosed the mix pulmonary infection of tuberculosis and the probable invasive aspergillus by chest CT and biopsy. The biopsy of the lower part of the trachea and the upper lobe of the right lung by fiberbronchoscope indicated that chronic inflammation of the mucosa with granulation formation, Chest CT scan shows: right supraclavicular fossa and right hilar and mediastinum multiple enlarged lymph nodes. The medication regimen consisted of rifapentine （0.6g 2 / week）, pyrazinamide （0.5 3/day） and isoniazid （0.3g 1 / day）. At the same time, the patients received chemotherapy according to the routine procedure: decitabine 15mg / m2 d1-5, aclamycin 10mg d3-6, cytarabine 10mg / m2 q12h d3-9, recombinant human granulocyte stimulating factor 200mg / m2 d0-9. Serum G test was 0.795ng/l and GM test was 0.93ng/l. He was suspected as pulmonary invasive aspergillosis, and a standard dose of voriconazole was used. The patient's condition continued to worsen. Chest CT was reexamined in 8 days after treatment. The blood concentration of voriconazole was monitored and the minimum concentration was less than 0.05ug/ml, then changed posaconazole (1200mg / d) for two weeks, the lung lesions were significantly improved (Fig1). The minimum plasma concentration of posaconazole was 0.58ug/ml, and the peak concentration was 0.76ug/ml (Fig2). Posaconazole was administered for a total of 10 weeks until the lung lesions disappeared. During this period, we did not observe the adverse reactions related to posaconazole, including clinical manifestations and laboratory indicators.
For the first time, we simultaneously use posaconazole and rifapentine for mixed infection treatment. We applied posaconazole and rifapentine to treat the mix infection. The patient's mixed infection treatment was successful, and lung CT showed that the lung nodules disappeared.
Conclusion: We recorded in detail the plasma concentration of posaconazole and rifapentine after combined use, explored the dose adjustment of posaconazole after combined use.

Keywords: hematological malignancies, Infection, Drug interaction

P-6

IoMT Platforms: Issues and functionalities

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The rapid development of mobile computing, wireless communication, sensor networks, and embedded systems has also resulted in health information systems approaching the era of pervasive computing. This has led to a constant growth of applications and devices (i.e., wearable devices) for providing new pervasive health services [1]. In the healthcare domain, IoT applications have led to the concept of the Internet of Medical Things (IoMT) [2]. IoMT-based platforms can empower the patient in the sense that they become self-aware of their health status [3]. In many cases, they even avoid or minimize the intervention of healthcare professionals. Some of IoMT platforms defined and proposed by researchers have been showed in Table 1. However, the number of IoMT devices is expected to grow considerably in the coming years and the heterogeneity present in different IoMT components (network interfaces, communication protocols, data structure, data semantics) will impose interoperability and privacy related challenges [10]. In this sense, a pervasive healthcare platform must be flexible enough to handle all these concepts. Therefore, the integration of IoMT solutions in an interoperable environment and the development of tools for the storage, processing, and widespread dissemination of IoMT data have become relevant. An IoMT platform must be adaptable in such a way that enable the use of existing communication protocols and the integration of new ones.. However, the lack of proposals for their integration with IoMT platforms [12] has caused health professionals to manage a patient's clinical history with distributed records provided by multiple sources. IoMT is one of the novel approaches for enhancements in current healthcare services.A modern pervasive healthcare platform should provide, at the minimum, functionalities for (a) Storage and management of clinical and demographic data in a standardized EHR in relation to their organization in a human-understandable approach; (b) A CTL mechanism for the integration of multiple IoMT solutions which consider heterogeneous communication protocols; (c) Management of patient clinical data by medical teams (creation, visualization, update and deletion of electronic records); and (d) Knowledge extraction and analytical studies to support clinical DSS services..

Keywords: Internet of Medical Things (IoMT), Pervasive Health, IoMT-based Platform

P-7

How to decision approach in fever of engraftment than infection

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A 18 years old new case of AML after partial remission in MRD evaluation, refer to transplant center for full match allogenic transplantation. He treated with FLASMA regimen and then Allogeneic transplant of his only sister.
In + 8 days of transplantation time her experience high grade fever unresponsive to wide spread antibacterial, anti-fungal, anti-viral drugs therapy.
In past history his mother explained prolonged and resistance fever to therapy in his induction of AML.
And we now must decide for this high grade fever not responsive to therapy of widespread above drugs in neutropenic patient.
He been confused and 3 days febrile , according in practical experience and of transplantation time he treated with corticosteroids.
After 24 hours he be alert with resolved fever.
Conclusion, gradually decisions for specific therapy in critical time is not simple and need preparation of a full past history and correct decide.

Keywords: Engraftment, time, treatment