T.C.

BAHÇEŞEHİR UNIVERSITY

INVESTIGATION OF HEMATOPOETIC STEM CELLS IN SPLEEN AND FETAL LIVER VIA α-CATULIN TRANSGENIC MICE MODEL

Master's Thesis

DENİZ ALTUNSU

İSTANBUL, 2019

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GRADUATE SCHOOL OF HEALTH SCIENCES NEUROSCIENCE DEPARTMENT

Supervisor: Assist. Prof. Dr. Timuçin AVŞAR

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Thesis Title: Investigation of Hematopoietic Stem Cells in Spleen and Bone Marrow via α-catulin Transgenic Mice Model
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Thesis Defense Date : 29.05.2019

The thesis has been approved by Graduate School of Health Sciences.

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Signature

This is to certify that we have read this thesis and we find it fully adequate in scope, quality and content, as a thesis for degree of Master of Arts.

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PREFACE

Above all, I would like to thank my supervisor Assist. Prof. Dr. Timuçin AVŞAR for academic and spiritual supports. In addition, he has professionally guided my first steps in the laboratory.

I would like to thank my other supervisor Assoc. Prof. Dr. Melih ACAR for everything. I am very grateful to him for all his guidance.

I would like to give my deepest thanks to Bahçesehir University Neuroscience Laboratory team helped me along the project.

I would like to give my thankfulness to my friend Esat Kurt for all support and love.

And last, but not least, I would like to give my thankfulness to my father Galip Altunsu, my sister Berrak Altunsu and my mother Süreyya Altunsu for all love and supports they gave along my whole life.

ABSTRACT

INVESTIGATION OF HEMATOPOIETIC STEM CELL IN SPLEEN AND FETAL LIVER VIA A-CATULIN TRANSGENIC MICE MODEL

Deniz ALTUNSU

Neuroscience Master Program

Thesis Supervisor: Assist. Prof. Dr. Timuçin AVŞAR

May 2019, 63 pages

This study is based on hematopoietic stem cell transplantation by use of a-catulin transgenic mice model. HSCs can give rise to all blood stem cells which are reside in the bone marrow. HSCs are one of the most important topics because it plays significant role in treatment of blood cancers, malignant or non-malignant diseases and also some metabolic diseases. Bone marrow transplantation (BMT) is a common and grateful example of stem cell therapy. They have some capacity of hematopoietic stem cells (HSCs) to self-renew and differentiation. These characteristic features provide the production of all blood cell lineages and simultaneous maintenance of the stem cell pool, thereby maintaining lifelong homeostasis. Banked umbilical cord blood (CB), spleen and fetal liver are crucial and readily available stem cell resource. However, the number of hematopoietic stem cells and progenitor cells are very rare and low compared with bone marrow.

The biggest limitation in hematopoietic stem cells is cannot be multiplied outside the body. The universal challenge is the culture of HSCs outside their natural environment has proven difficult. It is therefore important to understand how HSC self-renewal, proliferation, and differentiation are integrated, which molecules participate in their regulation and how these could be modified for clinical benefit. If this problem is solved, mutations that cause disease in these stem cells can be corrected, and these cells are transported back to the patient. Hence blood diseases can be eliminated.

In this study, a-catulin transgenic mice were used for all transplantation experiment. These transgenic mice cannot express the α-catulin protein so that called knockout mice. This protein play important role in cytoskeletal. To observation of hscs, GFP were added instead of α-catulin protein. By use of the α-catulin-GFP mouse model, hematopoietic stem cells were separated from other blood cells. After the lethal dose radiation, the irradiated mice were transplanted with spleen and bone marrow cells and the phenotype were replaced to old. Finally, the gfp stem cell population in spleen or

bone marrow was examined by sorter machine. Previous studies have identified the location of stem cells in the bone marrow. Our study focused on examination of hscs in spleen and fetal liver via α-catulin transgenic mouse model.

Keywords: HSCs, HSC Transplantation, Transgenic mice, Blood disease, Stem cell therapy.

ÖZET

INVESTIGATION OF HEMATOPOIETIC STEM CELL IN SPLEEN AND FETAL LIVER VIA A-CATULIN TRANSGENIC MICE

Deniz Altunsu

Sinir Bilim Yüksek Lisans Program

Tez Danışmanı: Assist. Prof. Dr. Timuçin AVŞAR

Mayıs 2019, 63 sayfa

Bu çalışma da CD45 konjenik fare kullanarak hematopoetik kök hücre nakli yapılmıştır. Hematopoetik kök hücreler çok az ve nadir sayıda kemik iliğinde bulunur. Bu kök hücreler yüksek oranda bölünebilme ve farklılaşma yeteneğine sahip olup tüm kan hücrelerinin yapımında aktif rol oynarlar. Hematopoetik kök hücre ve bu hücrelerinin nakli bir çok hastalığın tedavisinde ya da hastalığın seyrini değiştirmek amacıyla kullanılmaya başladı. Böylece kan kanseri, ölümcül ya da ölümcül olmayan hastalıklarda ya da metabolik birçok hastalığın tedavisinde hematopoetik kök hücre nakli kullanılmaya başladı. Kemik iliği transplantasyonu en yaygın ve tatmin edici sonuçların alındığı kök hücre terapisidir.

Aynı zamanda bu kök hücreler limitsiz bölünebildiği gibi yüksek oranda kendilerini yenileme özelliklerine de sahiptir. Bu karakteristik özellikleriyle hem tüm kan hücrelerini oluşturur hem de hematopoetik sistemin dengede kalmasını sağlarlar. Hematopoetik kök hücreleri sadece kemik iliğinden değil dalak, embryonik hücrenin karaciğerinden, kordon kanından vs. farklı farklı yerlerden elde edilebilir. Tabiki bu kaynaklardaki kök hücre sayısı ve kalitesi kemik iliğiyle kıyaslanamaz.
Hematopoetik kök hücrelerin elde edilmesindeki en büyük zorluk bu hücrelerin vücut dışında kendini koruyamayıp, hemen farklılaşması. Eğer bu evrensel sorun çözülürse, birçok mutasyona bağlı kan hastalıklarının ya da metabolik hastalıkların tedavisi mümkün olabilir.

Bu çalışmada, transgenik fareler optimum ölümcül radyasyona maruz kalıp, hematopoetik sistem baskılanmış oluyor. Radyasyon sonrası farklı kök hücre kaynaklarını kullanarak hematopoetik sistemin yeniden aktifleşmesini, kendini yenilenmesini amaçlandı. Bu transgenik farelerden, membran proteinlerinden biri olan a-catulin proteinini ekspres eden gen bölgesi çıkartılmış olup yerine kök hücre analizinin daha kolay bir şekilde yapılması için gfp eklenmiştir. Öncelikle farklı radyasyon denemeleri yapılarak en iyi optimum ölümcül doza karar verildi. Eğer fareler over-doz olursa buna bağlı enfeksiyon vs. oluşabilir. Sonrasında ise farklı kaynaklardaki hematopoetik kök hücreleri kullanarak ve yine farklı sayılarda kök hücre nakli yaparak radyasyona maruz kalan farelerin hematopoetik sistemlerinin yeniden aktifleştirmesi hedeflendi.

Anahtar Kelimeler: Hematopoetik kök hücre, HKH transplantasyonu, transgenik fare, kan hastalığı, kök hücre tedavisi

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ABBREVIATIONS

AML	:	Acute myeloid leukemia
BFU-E	:	Burst forming unit erythroid
BM	:	Bone marrow
BMP	:	Bone morphogenetic protein
BMT	:	Bone marrow transplantation
BrdU	:	Bromodeoxyuridine
CB	:	Cord blood
CBT	:	Cord blood transplantation
CDKI	:	Cyclin-dependent kinase inhibitor
CFC	:	Colony forming cell
CFU-E	:	Colony forming unit erythroid
CFU-GM	:	Colony forming unit granulocyte/macrophage
CFU-S	:	Colony forming unit spleen
CLP	:	Common lymphoid progenitor
CMP	:	Common myeloid progenitor
CRU	:	Competitive repopulation unit
CXCR4	:	C-X-C chemokine receptor type 4
DNA	:	Deoxyribonucleic acid
ECM	:	Extracellular matrix
EPO	:	Erythropoietin
ESC	:	Embryonic stem cell
FACS	:	Fluorescence activated cell sorting
FGF	:	Fibroblast growth factor
FL	:	Fetal liver
G-CSF	:	Granulocyte colony-stimulating factor

GDF	:	Growth and differentiation factor
GFP	:	Green fluorescent protein
GMP	:	Granulocyte/macrophage progenitor
HCT	:	Hematopoietic cell transplantation
HLA	:	Human leukocyte antigen
HPC	:	Hematopoietic progenitor cell
HSC	:	Hematopoietic stem cell
HSPC	:	Hematopoietic stem- and progenitor cell
IGF	:	Insulin growth factor
IL	:	Interleukin
KO	:	Knockout
Lin	:	Lineage
LSK	:	Lin- Sca-1+ c-kit+
LTC-IC	:	Long-term culture initiating cell
LT-HSC	:	Long-term HSC
MAPK	:	Mitogen-activated protein kinase
MEP	:	Megakaryocyte/erythrocyte progenitor
MPP	:	Multipotent progenitor
PB	:	Peripheral blood
ROS	:	Reactive oxygen species
Sca-1	:	Stem cell antigen 1
SCF	:	Stem cell factor
SCID	:	Severe combined immunodeficiency
SDF-1	:	Stromal derived factor-1, also called CXCL12
ST-HSC	:	Short-term HSC
TPO	:	Thrombopoietin

SYMBOLS

dH2O	:	Distilled Water
EtBr	:	Ethidium Bromide
KCl	:	Potassium Chloride
MgCl2	:	Magnesium Chloride
MgSO4	:	Magnesium Sulfate
NaCl	:	Sodium Chloride
NaOH	:	Sodium Hydroxide
TRIS HCl	:	Trizma® Hydrochloride

1. INTRODUCTION

In this study depends on HSC transplantation and reconstitution of hematopoietic system after radiation therapy. Stem cells have high regenerative potential in different organs such as bone marrow, spleen, fetal liver and also cord blood.

One of the biggest difficulties in tissue regeneration is retrieving and successfully delivering the stem cells poses. This issue is the biggest limitation in clinically. The hscs have the inherent capability of intravenously infused acting as a seed and engraft their tissue. But the BM is an exception in this issue. Bone marrow makes the essence for a more than four-decades-long clinical usage of bone marrow transplantation. In addition, BMT was apparent that finding a proper donor within an acceptable time frame would be a limitation for this treatment modality at the beginning of BMT. Now, there are alternative sources of stem cells. They were provided to increase the donor pool. These sources are spleen, peripheral blood and also fetal liver.

HSCs are the most important treatment way for the malignant or non-malignant blood diseases. HSCs give rise to other blood cells and this process is called hematopoiesis. This happens in the red bone marrow but the specific location of these cells remains controversial. HSCs are very rare and few can be found in this section. The more healthy HSCs are required for some patients that have cancers of blood and bone marrow such as Meloma or Leukemia. Because it's the patient’s immune system is destroyed with radiation or chemotherapy before the transplantation. After the allogeneic HSCT, some infection or graft versus host diseases can occurre. In addition to that, HSCT is a dangerous procedure with lots of complications.

During the this study, green fluorescent protein (GFP) knock-in for the gene Ctnnal1 in mice (here after denoted as a-catulin-GFP) was used and a-catulin-GFP was expressed in only 0.02 percent of bone marrow hematopoietic cells, including almost all HSCs. approximately 30 percent of a-catulin GFP1c-kit1 cells give long-term multiline age reconstitution of lethally-irradiated mice. So that a-catulinGFP+ c-kit cells show hsc purity by the using of some markers (Acar, Kocherlakota et al. 2015). The purpose of

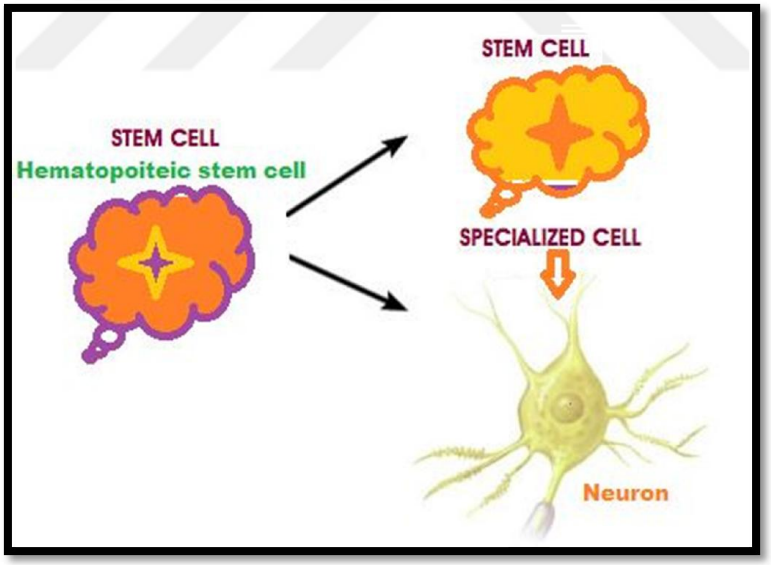
This study is the reconstitution of hematopoietic system after the radiation. The following purpose is the measuring multi-lineage reconstitution of hematopoiesis in transplanted-recipient mice This experiment results are observed at least four months and after the allo-HSCT, irradiated mice that shows 500.000 and 1 million cells have survived and shifted own phenotype so we obtained good results for competitive reconstitution.

2. LITERATURE REVIEW

2.1 STEM AND PROGENITOR CELLS

A stem cell has individual ability to develop into functional cell types in the body. Stem cells can be used to replace cells and tissues that have been damaged or lost due to radiation, chemotherapy, or disease. A human body is produced many types of cells. Most cells are specialized to execute particular functions. For example, red blood cells that transport oxygen around our bodies in the blood, but they are unable to divide. Stem cells provide the generation of new cells for the body as it grows, and replace specialized cells that are damaged or lost. They have two unique features that enable them to do this: They can divide over and over again to produce new cells.

Figure 2.1: Stem cell and specialized cells

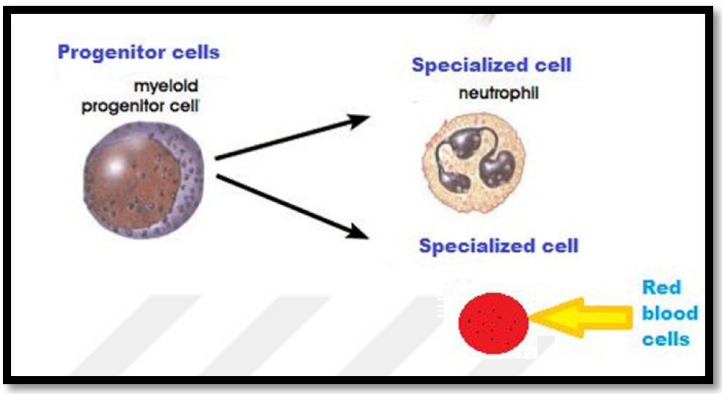


Reference: This figure was created by Deniz ALTUNSU.

While they divide, they can change into the other types of cells that make up the body. But such cells are basic for development through childhood, remaining pools of adult stem cells are hypothesized to be the source of the usually limited tissue regeneration and reconstruction that occurs in adults because of that tumor cells and embryonic stem

cells are not like adult stem cells, they are not immortal, and show reducing telomere length while increasing age (Warburton, Perin et al. 2008).

Figure 2.2: Progenitor cells and specialized cells.



Reference: This figure was created by Deniz ALTUNSU.

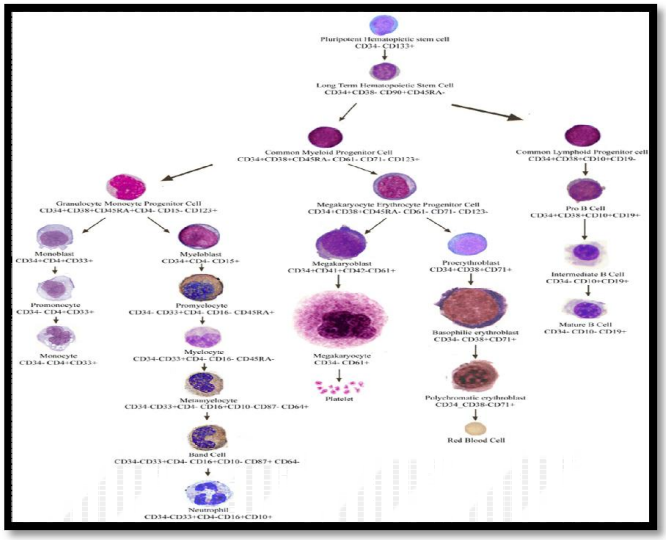
2.1 HSCs

The main idea of a common ancestor capable of generating the entire spectrum of hematopoietic cells in a cellular hierarchy was first proposed at the transition from the 19th to the 20th century. The generation of blood cells is rely on the activity of a rare and few stem cell population that normally resides within the bone marrow (BM) of the organism (Kohlscheen, Schenk et al. 2019)**.** These hematopoietic stem cells (HSCs) can ability to both self-renew and differentiate to all blood cells in addition to ensuring this lifelong hematopoiesis. Hematopoiesis is the process by which the cellular components of blood are frequently renewed throughout the endurance of an organism (Pinho and Frenette 2019). LTRCs and STRCs are identified stem cells. LTRCs have highly potent effect of producing all other blood cells for the whole life span of an individual organism (Morrison and Weissman 1994). During the limited period of life of the organism, STRCs provide reconstitution of the myeloid or lymphoid cells. (Morrison and Weissman 1994). While the hematopoiesis, stem cells have the ability to proliferation and sequential differentiation and all processes are followed by a gradual loss in self-renewal potential and the ability to generate mature cells.

According to the previous study, the numbers of stem cells are very low and they divide relatively infrequently. Consequently, the transit population of occupied progenitors is responsible for most of the cell amplification that takes place to save blood cell production (Abkowitz, Catlin et al. 2002).

The hierarchy in hematopoietic tissue is as figure. For adult bone marrow, MPP are the first progeny of HSCs and MMP have a high pluripotent differentiation capacity, but lack of self-renewal ability compared with hscs (Adolfsson, Borge et al. 2001). The CMP and the CLP is known that common precursors descend from the MPP. Both MEP and GMP derived from CMP. It is suggest that during commitment, the destiny of multiline-age progenitors converts restricted in a stepwise and invariable method. Some different lineage options have been described, mainly in B cell/macrophage and T-cell progenitor and CMP (Allman, Sambandam et al. 2003). These unexpected conclusions might be defined by a stochastic model during the initial phase of commitment. According to this paradigm, there is a good appointment of heterogeneity between progenitors with a continuum of overlapping potentials. Of course, any combinations are less probable to occur such as B cell and macrophages (Allman, Sambandam et al. 2003) whereas others are much more beneficial and are more likely to happen such as granulocyte and macrophage.

Figure 2.3: Hsc map.



Reference: Hsc mapping is referred by (Attar 2014).

2.1.1 Stem Cell Niche

BM supplies an environment for HSCs so that stromal cells, some of the fat-containing cells, sinusoidal blood vessels and the extracellular matrix can communicate with each other. The blood sinusoids are placed in the bone marrow. It provides differentiation for progenitors and mobilization for mature cells. The niches that called specific microenvironments for the stem cell reside in situ. At the beginning of the 1970s, the stem cell niche was first introduced for the human hematopoietic system (Schofield 1978). This niche regulates the fate of the stem cells and manages the scale between commitment and self-renewal. It can be conceptually divided into two parts. These are osteoplastic zone and vascular zone.

One of the most significant elements of adult BM niche is osteoblasts. They are consist of bone cells and derived from mesenchymal stem cells (Qin, Wang et al. 2016). They are heterogeneous population because of immature cells and mature cells, which supply to the bone formation (Calvi, Adams et al. 2003). It has been proved that the number of HSCs is depended on the number of osteoblasts (Visnjic, Kalajzic et al. 2004). The cells within the niche generate factors because of inhibiting differentiation of HSCs and

maintaining them within the micro environmental compartment. Some of the adhesion molecules which are N-cadherin and betha1-integrin play a pivotal role in the intercommunication between HSCs and osteoblast(Marjon, Termini et al. 2016). HSCs express usually a4b1 and a5b1integrins and they bind to fibronectin so that it promotes adhesion to BM stromal cells(Joshi, Goihberg et al. 2017). Some proteins need for localization of HSCs to hematopoietic organs is the chemokine. They are included in stem cell homing(Naderi-Meshkin, Bahrami et al. 2015). The most important chemokine which is the stromal derived factor-1(SDF-1) and its receptor CXCR4 is used for localization of HSCs in BM (Zhang, Ren et al. 2014). For example, leukocyte cell adhesion molecule, osteopontin, bone morphogenetic protein, the Notch ligand gamma and angiopoietins expressed on osteoblasts and it plays a role in bone marrow microenvironment interactions(Heath, Cohn et al. 2019).

According to the previous study embryonic and adult life in vascular niche can be different. Although interactions during adult life are not as greatly known, embryonic life entirely studied strong interactions between HSCs and the developing vascular system take place. Throughout embryogenesis, hematopoietic and endothelial cells are originated from common progenitors which means that the hemangioblasts (Choi, Kennedy et al. 1998). The ligand-receptor signaling resides between HSCs and endothelial cells. For instance, HSCs secrete angiopoietin-1 and that molecule induces angiogenesis(Takakura, Huang et al. 1998). Vascular endothelial cells contribute inductive signals for organ development. This condition is not as obvious for adult life. Because the vascular niche is described as a place for stem cell mobilization or proliferation and differentiation of progenitors in adult BM(Heissig, Ohki et al. 2005). It’s hypothesized that progenitor cells that surrounding the stem cells can be estimated a third type of niche cells(Gerber, Malik et al. 2002). In addition, progenitor cells suppress stem cell proliferation, alike to lateral inhibition happened in neural cells. If the progenitor number is reduced, HSCs are released and divide(Gerber, Malik et al. 2002).

2.2.2 HSCS Engraftment, Homing, Seeding

Successful transplantation of HSCs is more complex process. This process consists of homing, seeding and engraftment. Although stem cell niche is a crucial process, the niche is provided that normally hosts strong and multiline age differentiation of hscs (Berrios, Dooner et al. 2001, Kucia, Reca et al. 2005). Following transplantation, hscs are introduced into systemic circulation distribute in accordance with hydrostatic and chemotactic determinants(Sackstein 2016). Multiple interactions between HSCs and endothelial cells have controlled that process. Some adhesion molecules such as integrin, sialomucins, and CD44 isoforms are expressed by HSCs(Chute 2006).

Selections that E- and P-selection is expressed by Endothelium (Lapidot, Dar et al. 2005). First of all, the cell is attached to the endothelial wall, chemo attractants namely stromal-derived factor-1 (SDF-1) influence an intracellular signaling cascade and then activation of integrin have happened(Yang, Wang et al. 2018). Integrin form firm adhesion via binding to counter-receptors on the endothelial cell surface (Chute 2006). A powerful role acts interaction between a4b1 integrin, VLA-4 and VCAM-1 (Karantanou, Godavarthy et al. 2018). Following HSCs adhesion and rolling, the next step is transmigration of hsc into the bone marrow vessels. SDF-1 play important role in this process. SDF-1 is expressed on endothelial cells and bone marrow cells (Orschell-Traycoff, Hiatt et al. 2000). Hscs usually express CXCR4 which is a receptor for SDF-1(Zhang, Ren et al. 2014). After transplantation, their mutual interaction is central axis governing stem cell homing in the bone marrow.

Seeding of HSCs is defined via adhesive communication with the stromal. Preferably selective seeding because of single molecular pair, interaction with bone marrow stromal is linked by speedy phenotypic differences in expression of cell molecules while the first days after transplantation(Stein, Yaniv et al. 2005). He assumed that cells take advantage of the pre-existing repertoire of adhesion molecules just for the first steps of homing and early seeding. As soon as HSCs express additional molecules and this process that promotes engraftment. Engraftment is proliferation and differentiation of donor HSCs. There are two different types of niches. The one niches provide to

maintain functional quiescence of cells for a long term and other niches help early dividing of cells (Askenasy, Yolcu et al. 2003). After some time, the site of activity changes towards the more middle regions of the bone marrow.

In conclusion, the correct association of seeding and engraftment in existence shows the cooperatively of stromal guidelines and both, versatile and inherent HSCs responsiveness in the dynamic of early engraftment.

2.2.3 Mobilization of Hscs

According to the previous study, the interactive network of adhesive interactions have controlled the retention of HSCs within hematopoietic organs (Gumbiner 1996, Verfaillie 1998). The numbers of HSCs circulate in the peripheral blood (PB) is very low under the normal conditions(Zhang, Ren et al. 2014), this event is the outcome of dynamic equilibrium among efflux from the BM into the PB and recapture or rehoming of HSCs into the BM (Wright, Wagers et al. 2001). Mechanisms leading to HSCs mobilization are not completely understood compared with homing. The major roles of the adhesion molecules in the mobilization has appeared, like homing and retention(Lévesque, Hendy et al. 2002).

Some different mechanisms may provide to stem cell mobilization (Zhang, Ren et al. 2014). If stem cell expansion is inducted, stem cells can migrate to the peripheral blood and spleen(Salam, Salem et al. 2017). Such mechanism relies on hematopoietic growth factors which are affected first stem cell growth such as stem cell growth factor (SCGF) and FIT-3 ligand (FL). Another way for mobilization is the treatment of IL-8 (Laterveer, Zijlmans et al. 1996). In addition to that, G-CSF has induced mobilization of HSCs by the role of metalloproteinase(Zhang, Ren et al. 2014). Both clinically and experimentally, mobilization of HSCs is induced by some of the cytokines, such as granulocyte colony-stimulating factor (G-CSF or GM-CSF), AMD3100(Miller, Nakamichi et al. 2016). G-CSF play important role in the mobilization of HSCs, but its effect is dependent on time and dose, in addition, G-CSF is rapidly reconstituted just for neutrophil which is promote the increase of HSCs numbers in PB with a maximum

between 3-6 days after G-CSF treatment (Sundaramoorthy, Wang et al. 2017). According to the studies, mobilization of HSCs induced by chemotherapeutic factors is limited, happening in mice about day 6 while the recovery phase that follows the leucopenia induced by chemotherapy. If G-CSF is added to myeloid suppressive drugs, HSCs mobilization can be increased (Sato, Sawada et al. 1994). Some data shows that the mobilization of HSCs is correlated with expanded production of proteases inside the bone marrow space. These enzymes degrade the extracellular matrix (Lataillade, Domenech et al. 2004). Mobilization induced by G-CSF is linked with increased SDF-1 degradation by elastase (Petit, Szyper-Kravitz et al. 2002) and matrix metalloproteinase 9 (MMP-9)(Zhang, Ren et al. 2014). This result demonstrated that after G-CSF treatment, proteolysis of the extracellular domain of CXCR4 has happened through the working of elastase, parallel to the cleavage of VCAM-1. Degradation of CXCR4 is play important role in internalization and HSCs mobilization(Zhang, Ren et al. 2014). Hence, immediately preventing the communication between SDF-1/CXCR4 alone can trigger HSCs mobilization from BM to the PB (Liles, Broxmeyer et al. 2003). After the administration of monoclonal antibodies that against VLA-4 or VCAM-1 may effect mobilization of HSCs needing signaling throughout the c-kit/c-kit ligand pathway(Papayannopoulou and Nakamoto 1993, Craddock, Nakamoto et al. 1997).

Nowadays, some people believe that HSCs mobilization induced by G-CSF, cytotoxic agents and by chemokine play important role in neutrophils(Domingues, Nilsson et al. 2017). During mobilization by G-CSF, neutrophils play a pivotal role in that process with their huge degranulation(Sundaramoorthy, Wang et al. 2017). When G-CSF is the disrupted adhesive communication between VCAM-1 and VLA-4 lead to mobilization, which means that consequence of VCAM-1 break by neutrophil proteases such as neutrophil elastase (NE) and catepsin G (CG), delivered by neutrophils activation in the extravascular section of the BM following G-CSF treatment. Reinfusion of mature neutrophils restores mobilization in response to G-CSF or chemokines (Aiuti, Tavian et al. 1999). It also shows that result of activation of neutrophils within the BM, transformation of the BM extravascular compartment can happen toward a highly proteolytic environment and that is a general hallmark of mobilization induced by G-CSF or chemotherapy(Levesque, Liu et al. 2004, Winkler and Levesque 2006).

2.2.4 Identification of hematopoietic stem cells and some functional markers

All stem cells are identified by the using of cell surface markers. Most usually, the HSCs are distinguished from each other without all lineage markers (Lin-). There are some interspecies variations. While the identification of human HSC via specific cell surface antigens, murine HSCs cannot analysis by the using of the same antigen.
In the experimental setting, the murine HSCs were first defined to be in c-Kit+ Thy1lo Lin- Sca-1hi population (Spangrude, Heimfeld et al. 1988). According to the presence of a cocktail of surface antigens LTRC, STRC as well as some progenitors can be selected. Recently, HSCs are identified via SLAM family markers. All HSCs were shown to be CD150+, CD48- (Yilmaz, Kiel et al. 2006). The LTRC is the most exciting fraction of bone marrow cells which are able to long-term repopulation and present within this Thy1lo fraction (Christensen and Weissman 2001). CD34 was used to distinguish stem cells in bone marrow for a long time in human, only just was found that unstimulated LTRC in BM are CD34 negative(Bhatia, Bonnet et al. 1998). CD34 is expressed on only 0.5-5% of human bone marrow cells. If HSCs are identified into the peripheral blood, CD34 is still used. However, the most usually used stem cell marker for human BM is CD133 antigen.

In addition, the most intense population of HSCs resides within the SP fraction. This population is described according to the extrusion of Hoechst 3342 from the cells and is mainly improved for HSCs. The other markers may be utilized to the classification of HSCs, such as CD10, CD7, IL-3R-a-low(Ziegler, Valtieri et al. 1999). Although using of a large antigen identifying cocktail, the HSCs population remains can be heterogeneous. The using markers are not crucial for stem cell function and consequently, the expression of these markers might not immediately associate with stem cell potential(Matsuoka, Ebihara et al. 2001). Stem cells that population of cells has stochastic gene transcription that is likely to create phenotypically heterogeneous cells with same functional ability. After transplantation, some assays are used for hscs ability to give rise to long-term engraftment in the lymphoid and myeloid-erythroid lineages in a lethally irradiated host. Colony forming units (CFU-S) have the capacity to the generation of colonies in the spleen of a mouse eight to twelve days after transplantation, which included cells of various lineages. Secondary irradiated host

could be repopulated by a small part of those colonies(Till and McCulloch 1961). Now the long-term repopulating capacity of CFU-S is regarding Day-12 CFU-S which is derived from first MPP and Day-8 CFU-S are derived from late myelo-erythroid active progenitors (MEP)(Lee, Zhou et al. 2017). During the survival, proliferation, and differentiation of HSCs the bone marrow microenvironment may selectively support this process(Acar, Kocherlakota et al. 2015). By the using of in vivo competitive repopulation assays, HSCs can be evaluated after transplantation into the irradiated congenic host and long-term engraftment of HSCs can be confirmed by secondary and tertiary transplantations. Transplantation of human stem cells into xenogeneic hosts is used to detection of human HSCs into immunodeficient NOD/SCID or RAG-/RAG- mice and presence of human cells in the blood and hematopoietic tissue is identified(Wang, Ge et al. 2018). All differentiated human hematopoietic cell lineages should be identified. All transplantation assays are related to the future potential of stem cells and this is a weak point. So the nature of hscs can be assumed only retrospectively. This result shows that these assays do not declare anything about the behavior of a single cell, but about the probabilistic behavior of a cell population. In addition, hscs have ability to self-renewal and multipotent differentiation potential, but also express some important molecules for homing to the bone marrow of the hosts. Lately, the homing interference has been rejected through direct cell transfer of HSCs into the bone marrow cavity by intra-bone injection(Guo, Huang et al. 2016).

2.3 STEM CELL-BASED THERAPY

Stem cells are superior targets for regenerative medicine because they can support and repair tissue. The fluid nature of the hematopoietic system provides easy delivery of HSC to their host tissue so that HSCs are generated. It is the prototype of organ renewing stem cells and the model for other stem cell-based therapies. Transplantation of HSCs is the most common example of stem cell therapy. HSCT provides to treat hematopoietic malignancies such as leukemia, lymphomas and immunodeficiency. HSC transplantation was first proposed in the late 1950's and but clinical success and following using of that came with the discovery of the human leukocyte antigen (HLA) method and the choice of immunologically compatible donors at the beginning of the

70’s (Thomas 1999). The basic purpose of cellular therapy is the treatment with radio and/or chemotherapy to disrupt the patient’s own hematopoietic system and then followed by infusion of new stem and progenitor cells which increasingly reconstitute the hematopoiesis. Time to neutrophil recovery (TNR) or immune reconstitution can be called as a time to donor engraftment and it is a crucial period as it leaves the patient sensitive to infection with increased risk of transplant-related mortality. HCT can either be autologous or allogeneic. Autologous also called re-infusing the patient’s own HSCs and allogeneic also called involving a donor, which in turn can be associated or not. The success of allogeneic transplantations hugely depends on the immunological compatibility between donor and recipient tissues as negotiated by the HLA genes, and the associated with of graft-versus-host disease (GVHD) and graft-versus-leukemia (GVL) (Copelan 2006) and (Kolb 2008). The most commonly known that BM is used for all stem cell transplantation as the single source of HSCs. Today, HSCs are obtained from PB which is more commonly used (Körbling and Freireich 2011). Mobilization of hematopoietic stem and progenitor cells (HSPCs) from bone marrow into the blood circulation has been popularly used for hematopoietic transplantation. However, some of cytokine or small molecules are used in mobilization of HSPC. These new mobilizing agents are provide to increase the number of stem cells in peripheral blood for effective reconstitution of hematopoiesis (Zhang, Ren et al. 2014). These molecules inhibit binding of the homing molecule CXCL12 to its receptor CXCR4 (Broxmeyer, Orschell et al. 2005). The third stem cell source is a CB and it was introduced in 1988. While the choice of HSC source, depends on the needs of each individual patient. Because engraftment potential, kinetics, immunogenic characteristics, as well as development and severity of GVHD are different for each patient (Körbling and Anderlini 2001, Anasetti, Aversa et al. 2012).

The goal of stem cell therapy is based on the safe and efficient genetic engineering of HSCs. So it can be a potential solution to a variety of hereditary and acquired disorders(Rivière, Dunbar et al. 2012). Another method is gene therapy. It is used for the correction of a defective gene and thus the underlying monogenetic disease. It is obtained by means of ex vivo transduction of patient cells with viral vectors that have

the therapeutic gene and subsequent autologous HCT. The retroviral vectors are stably integrated into the hosts genome so that hierarchical of the hematopoietic system allows genetically altered HSCs to transmit their alteration to all mature blood cell types and theoretically provides life-long correction of the disease (Karlsson, Ooka et al. 2002). Gene therapy known that effective treatment method for severe combined immunodeficiency (SCID) and thalassemia and it has shown success with regard to therapeutic efficacy (Cavazzana-Calvo, Hacein-Bey et al. 2000, Gaspar, Cooray et al. 2011). However, first-generation retroviral vectors were observed by toxicity and resulted in leukemic transformation due to insertion activation of proto-oncogenes (Hacein-Bey-Abina, Von Kalle et al. 2003, Hacein-Bey-Abina, Von Kalle et al. 2003, Ott, Schmidt et al. 2006). These weaknesses seriously reduced research and clinical trials at the beginning of the 21st century and started to successfully apply HSC gene therapy.

According to scientist stem cell engineering implicates complicated biological difficulties. These challenges are the collection of patient cells for ex vivo administration, effective transduction of self-renewing HSCs to ensure gene delivery as well as polyclonal hematopoiesis, and relevant vector design to decrease the risk of insertional mutagenesis. To solve these problems should be focused on improvements in vector design and technologies for targeted gene delivery so that it will increasingly re-setup the hopeful potential of gene therapy (Rivière, Dunbar et al. 2012). If HSPCs successfully grow at the outside of their natural environment, gene modification and gene therapy approaches will be good.

Direct reprogramming of somatic cells to induced pluripotent stem (iPS) cells are the most crucial field for regenerative medicine and for personally cell therapies. The combination of pluripotent cells and embryonic stem cells (ESCs) are also called their prototype. Thus they have the ability to differentiate into any of the three embryonic germ layers. They have great potential for tissue regeneration because they maintain the capacity to compose all cell types of the body. Shinya Yamanaka showed four transcription factors which are c-Myc, Klf4, Oct4, and Sox2 was adequate to reprogram terminally differentiated murine fibroblasts into cells with ESC-like properties in 2006

(Takahashi and Yamanaka 2006). After this invention, reprogramming technology has become to improved quickly, iPS cell lines derived adult and neonatal tissues in the mice and the human system was generated(Broxmeyer, Lee et al. 2011). In addition to iPS, personalized medicine has been a hot topic and then the creation of patient-specific iPS cells, enabling disease modeling and in vitro drug screening studies has started (Robinton and Daley 2012). After the combination of reprogramming, gene repair, and tissue replacement therapy, animal models of sickle-cell anemia and Parkinson's disease has been created(Hanna, Wernig et al. 2007, Wernig, Zhao et al. 2008). These studies confirm that even though far from clinical training, reprogramming and iPS cell technology have huge potential to model and ultimately treat human disease. Finally, the realistic therapeutic potential and safety of iPS cells and their subtle differences to ESCs are not clear still(Anasetti, Logan et al. 2012, Robinton and Daley 2012).

2.3.1 Allo-hsc transplantation and origin

Firstly, the importance of bone marrow cells was found in the 1950s when it was mentioned that intravenous injection of bone marrow cells to irradiated mice because of the generation of their blood cell (Rekers, Coulter et al. 1950). The research area developed rapidly and the first report that allo-HSCT in humans described in 1957 so that procedure has created as we know it today (Thomas, Lochte Jr et al. 1957). Allogeneic transplantation which means that hematopoietic system of a patient is reconstituted by an intravenous admixture of hematopoietic stem cells derived from a relevant or an irrelevant donor, but autologous HSCT is called recipient´s own stem cells are re-injected.

2.3.2 Donor

The using of allo-HSCT can be limited because of the availability of a suitable donor. While the choice of a donor, some principle must be such as tissue compatibility or histocompatibility antigens located on chromosome 6. A well-matched donor should be at least 9 of the 10 alleles of the human leukocyte antigen (HLA) system which encodes the major histocompatibility complex (MHC) proteins9, 10. An absolute HLA-match

system consists of 10/10 alleles and can be found for around half of the patients including Western European ancestry but 20–30% of people can be matched for 9/10 alleles is usually available(Tiercy 2016).

The sibling is always good choosing for transplantation. Beside that well HLA-match can be 25% among the sibling. If there is no matched sibling donor, or it is not most ideal for transplantation, international unrelated donor registries may be searched for matched unrelated donor (MUD). If a matched donor cannot be found during the pleasing time, doctors can evaluate mismatched (haploidentical) related or unrelated donor. The safe use of haploidentical donors can be used by modern graft processing technologies, and particularly familial donors are often readily accessible.

2.3.3 Conditioning regimens

Before the transplantation, donor stem cells should be served with a conditioning regimen. While the choice of conditioning, disease indication is importantly based. In malignant disease, myeloablative conditioning (MAC) that destroys the host´s hematopoiesis without casual recovery is usually chosen for destroying minimal residual disease.

2.3.3.1 TBI

Total body irradiation (TBI) destroys minimal residual disease (MRD) and is immunosuppressive. There are some advantages of TBI. These are independence from drug immersion, metabolism or transport across the blood–brain barrier. TBI is usually coupled with a chemotherapeutic agent. The most common agents can be cyclophosphamide (Cy), etoposide or cytarabine (ARA-C). TBI is played an important role in the preparative regimens for malignant disease. TBI was often performed as a single portion TBI (sTBI) of 10–12 Gy. sTBI has been replaced by less toxic fractionated TBI (fTBI) because of high toxicity. Patients are also often given fTBI as 10–12 Gy, but radiation is divided into several fractions as 2–4 Gy over a time of two or three following days. In the treatment of childhood AML, TBI has been replaced by Bu. But in ALL, it cannot decide to whether TBI can be replaced by chemotherapy-based

conditioning regimens externally compromising survival(Willasch, Peters et al. 2017).

2.3.3.2 Elekta synergy

Elekta Synergy can be first high-level digital linear accelerator to insert soft tissue volumetric Image Guided Radiation Therapy. There are lots of advantages of this machine. Such can be excellent guidance tools; imaging of thin tissue at the time of treatment supports efficient verification of the tumor and critical structure position, giving increased confidence in dose placement.

Figure 2.4: Elekta synergy.

Reference: Elekta synergy (online) https://www.elekta.com/dam/jcr:e50d9ac2-e00f-45a4-bdac-f1d8943c3c22/Elekta-Synergy-Brochure.pdf [17.04.2019]

2.3.3.3. Chemotherapy based myeloablative conditioning regimens

As the early 1980s, Busulphan has been proposed as the myeloablative option to TBI (Atilla, Atilla et al. 2017). It is usually administered in combination with Cyclophosphamide (Atilla, Atilla et al. 2017). The oral treatment of Bu has a highly changing bioavailability but by applying intravenous treatment with a pharmacokinetic directed dosing the systemic exposure can be more quickly checked. The systemic exposure of Bu has a powerful relationship with the regimen associated beside severe

unfavorable results (Andersson, Thall et al. 2002). Myeloablative options to Bu because of less toxic profiles which is particularly in non-malignant disease. Treosulfan, trophosphamide, melphalan and thiotepa and an antimetabolite fludarabine are alkylating agents and they have been used in multiple combinations. Patients that severe aplastic anemia is usually treated with Cy-based conditioning regimens, sometimes in combination with fludarabine and/or a low dose of TBI.

2.3.3.4. Reduced intensity conditioning regimens

Reduced-intensity conditioning regimen (RIC) may be applied when the patient may not tolerate full myeloablative conditioning which is in case of previous myeloablative therapy or severe infections. Alkylating agents in RIC regimens or TBI are usually decreased by one third or more so these regimens become less toxic. Fludarabine is usually the key agent and this agent is used in combination with average doses of alkylating agents like Bu. RIC regimens in malignant disease depend also on the graft-versus-leukemia impact for inhibiting relapse. Some studies show that the background from RIC in the treatment of childhood AML in combination with immunotherapy, it has been well tolerated(Zahler, Bhatia et al. 2016).

2.3.3.5. Non- myeloablative conditioning regimens

Non-myeloablative regimens are rarely used in children, the exception of SAA. The non-myeloablative (NMA) regimens frequently include TBI in low doses (<2 Gy) fused with fludarabine or Cy (Servais, Baron et al. 2011). NMA suppresses the immune system because of engraftment but it does not destroy host hematopoiesis, thus providing the hematopoiesis to reconstitute shooting, and at engraftment mixed chimerism is required(Satwani, Morris et al. 2008).

2.4 MICE STRAIN

2.4.1 Congenic mice

Congenic mice are actually inbred mice which have just one exception. Congenic mice have different alleles at a specific gene. The purpose of that mice is to express the target allele which is found in one strain on the genetic background of another strain. These mice are created by first crossing two distinct inbred strains of mice, one strain contains the allele of interest and another that has the targeted genetic background. The offspring are genotyped because of distinguishing mice with the allele of interest and these mice are backcrossed to the inbred strain with the targeted genetic background. The next offspring with the allele of interest is repeatedly selected and mated to the original targeted background again. This process is replicated for 5-10 times, and the allele of interest will be on the targeted genetic background at the end of the 10th generation.

2.4.2 CD45.1/2 congenic mice

CD45.2 is a CD45 alloantigen which is expressed by Ly5.2 bearing mouse strains such can be BALB/c, CBA/Ca, CBA/J, and C57BL. CD45 comes from protein tyrosine phosphatase (PTP) family which is a 180-240 kD glycoprotein expressed on all hematopoietic cells exception of mature erythrocytes and platelets(Szelinski, Fleischer et al. 2018). It plays an important role in signal transduction induced by T and B cell antigen receptor engagement, cytokine signaling, and Fc receptor stimulation(Szodoray, Stanford et al. 2016). CD45 can exist in multiple forms and allelic forms CD45.1 (Ly-5.1) and CD45.2 (Ly-5.2)(Acar, Kocherlakota et al. 2015). This allele forms resolution of donor and recipient cells is important for gene therapy or bone marrow transplantation. As a result, CD45 recognizes all leukocytes of mouse strains.

All congenic mice is used for immunological studies. The most common congenic mice are CD45.1 and CD45.2. These mice are on the C57BL/6 genetic background. The one strain expresses the CD45.1 allele and the other expresses the CD45.2 allele at the same time.CD45 is a protein tyrosine phosphatase receptor expressed on all hematopoietic

cells but exception of mature erythrocytes.

2.4.3 CD45.1 and CD45.2 cells in Transplantation Experiment

CD45.1 and CD45.2 cells are generally used for transplantation experiment to observation of the donor and host cells. CD45.1 that leukocytes expressing is transferred into wild-type C57BL/6 mice and then detected by the using of CD45.1 expression. Cells of CD45.1 and CD45.2 can be identified using specific cell surface antibodies which recognize each form of CD45. CD45.1 specific antibody does not interact with leukocytes or mouse other cells expressing the CD45.2 alloantigen. CD45.1 bone marrow cells derived from the identical background so the host accepts it and allows the donor cells to circulate and develop in the context of the host hematopoiesis. If CD45.1 bone marrow cells from SJL/J mice are transferred to C57BL/6 host mice, the host rejects the donor cells because of differences in two strains of mice. Most studies show that the donor cells come from genetically engineered mice to investigate the role of the deleted or added gene in the wild-type mice. Some cells derived from knockout mice on a CD45.1 background could be mixed with CD45.2 wild-type cells to understand the role of the gene in the development of the cell lineage fate and decisions in a wild-type environment.

3. MATERIALS AND METHODS

3.1 MATERIALS

3.1.1. Chemicals

Table 3.1: List of chemical

Chemical Product	**Trademark**	**Catalog No.**
1 Kb plus DNA ladder	ThermoFisher	LSG-SM1331
6X Loading dye	ThermoFisher	LSG-SM1331
Absolute ethanol	Sigma-Aldrich	32221-2.5L
Agarose	Biomax	HS-800
APC anti-mouse CD45.2 Antibody	BIOLEGEND	109813
Boric acid	VWR	33601.261-500G
Dimethyl sulfoxide	Amresco	WN182-10ML
Ethidium bromide	VWR	0492-5G
Ethylenediaminetetraacetic acid disodium salt dihydrate	VWR	33600.267-500G
HBSS	Gibco	
Magnesium chloride 25mM	New England BioLabs	M0270L

Magnesium sulfate	Sigma-Aldrich	M7506-1KG
PE anti-mouse CD45.1	BIOLEGEND	110707
Phosphate buffered saline	Gibco	70011-036
Potassium chloride	Sigma-Aldrich	12636-1KG
Q5® high fidelity DNA polymerase	New England BioLabs	M0491S
Sodium chloride	Sigma-Aldrich	31434-1KG-R
Sodium hydroxide	DAEJUNG	7570-4100
Sterile water(molecular grade)	MULTICELL	809-115-CL
Taq 2X master mix	New England BioLabs	M0270L
Trizma® base	Sigma-Aldrich	T6066-1KG
Trizma® hydrochloride	SIGMA ALDRICH	T5941-500G
Tryptone	VWR	84610.05

References: This table was prepared by Deniz ALTUNSU

3.1.2 Equipments

All equipments used in this research are listed in Table 3.2.

Table 3.2: List of equipments

Equipment	Trademark
ChemiDoc MP Imaging system	BIO-RAD, USA
Digital heat block	Benchmark, USA
Digital scale	ISOLAB, Germany
DM2000 LED light microscope	Leica, Germany
FACS calibur	E97600376, USA
HI-2211 pH/ORP meter	HANNA instruments, USA
Microcentrifuge® 16	BECKMAN COULTER, USA
MicroPulser electroporator	BIO-RAD, USA
MX-S vortex	SCILOGEX, UK
nanoPAC-300	Cleaver Scientific, UK
PURELAB Option-Q	ELGA, UK
SL 40-FR centrifuge	ThermoFisher, USA
Sub-Cell® GT	BIO-RAD, USA
T100 Thermal cycler	BIO-RAD, USA

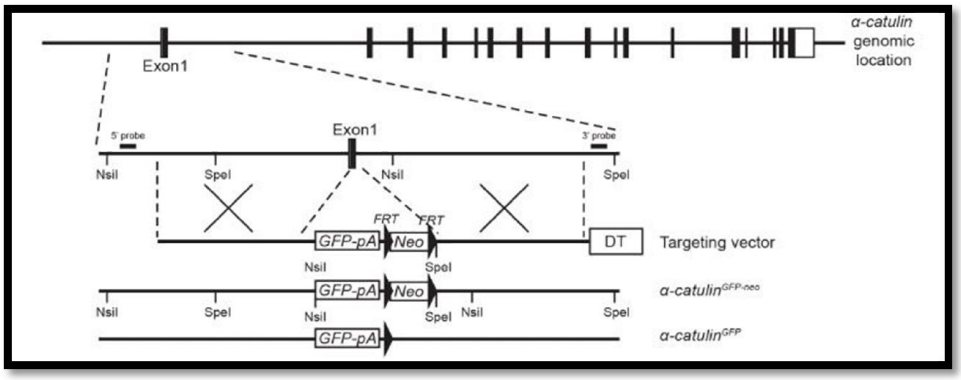
References: This table was prepared by Deniz ALTUNSU

3.2 METHODS

3.2.1 Mice

The main idea is generation of a-catulin GFP allele via recombineering. Linearized targeting vector was introduced into Bruce4 ES cells (Acar, Kocherlakota et al. 2015). Targeted ES cell clones were recognized with the Southern blotting technique and injected into C57Bl/6-Tyrc-2J blastocysts(Acar, Kocherlakota et al. 2015). The resulting chimeric mice were hybridized with C57Bl/6-Tyrc-2J mice because of obtaining germ line transmission. Next, the Frt-Neo-Frt cassette inserted by the targeting vector which is removed via mating with FLPe mice(Acar, Kocherlakota et al. 2015). Those mice were again crossed onto a C57Bl/Ka setting and germ line transmission was checked with PCR. C57Bl/Ka-Thy-1.1(CD45.2) and C57Bl/Ka-Thy-1.2(CD45.1) mice were used in all experiment of transplantation. Male and female mice approximately 1 year old were used for all studies. All mice were housed at the Bahçeşehir University faculty of medicine. These mice were kept at 23–25 °C with a 12 h day/12 h dark cycle during all experiment, and their food and acidified water was autoclaved.

Figure 3.1: Generation of a-catulin transgenic mice model



Reference: (Acar, Kocherlakota et al. 2015) Deep imaging of bone marrow shows non-dividing stem cells are mainly perisinusoidal, Nature.

3.2.2 Radiation Injury Mouse Model

Adult CD45.1 and CD45.2 C57BL/6 mice were irradiated with 630 with two sided, 650, 525, 500 and 450 RAD min lethal dose by TBI using a ELEKTA Synergy (2012) irradiator at least 6 hours apart. Mice were irradiated on a rotating platform. The used mice are male and 1 year old.

3.2.3 Transplantation Experiments

For competitive transplantation experiments, WBM and spleen cells were transplanted by injection into the retro-orbital venous sinus of anaesthetized recipient mice. 500,000 recipient whole bone marrow (WBM) and 1 million spleen and WBM cells were transplanted along with the donor cells. Actually, Both 1×10^6 spleen and bone marrow cells (CD45.2) and 1×10^5 congenic spleen and bone marrow competitor cells (CD45.1) were transplanted into lethally irradiated CD45.1 mice.

3.2.4 HSC Isolation

Bone marrow cells were isolated from the long bones which are tibias and femurs or cells in spleen were isolated by injector in Ca21- and Mg21-free Hank's balanced salt solution (HBSS, Gibco) supplemented with 2% heat inactivated fetal bovine serum (Gibco).

Spleen cells were set up by smashing the spleen between two glass slides. Bone marrow and spleen cells were gently passed through a 25-gauge injection syringe then filtered using a 100 mm mesh for generation of single cell suspension. Viable cell number was calculated by counting manually with a hemocytometer.

3.2.4.1 HSC analyzing by BD FACS

BD FACS was used to identification of HSCs. The isolated stem cells from the tibia, femur, spleen and spinal cord were suspended in 1XHBBS+%2 FBS until they get single. These cells were centrifuged at 1450 RPM for 5 min and then pulled supernatant with suction. All tubes were filled with 2 ml of 1xHBSS+%2 FBS after counting. These

cells were stained with specific cell surface markers. These markers are CD 150- PE-Cy5for lymphocyte , CD48-AF700 for B cells, Macrophages, Monocytes, T cells, Lin-PE (Ter119 for mature erythrocytes, B220 for B-cells, GR1 for myeloid, CD3 for T cells), C-kit- APC-Cy7(CD117 for pluripotent hematopoietic progenitor cells) , Sca1 – APC for peripheral B lymphocytes and thymic and peripheral T lymphocytes . The stained cells were incubated for 20 minutes at + 4 ° C. All tubes were filled with 1X HBSS + 2% FBS for washing step and again they were centrifuged at 1450 RPM for 5min. After the centrifuge, supernatant was pulled via suction. After antibody staining, the cells were stained with the viability dyes 49,6-diamidino-2-phenylindole (DAPI, 10 mg/ml in 1XPBS) to exclude dead cells during flow cytometer. DAPI of 1ml was distributed to each tube with different pipette tips. Analysis tubes were storaged at +4C, make sure the tubes are closed. Before the analysis, tubes were vortexed.

3.2.5 Competitive Reconstitution Assays in Irradiated Mice

Adult recipient mice were treated with lethal dose of radiation via an ELEKTA Synergy (2012) irradiator. ALL hsc cells were injected into the retro-orbital venous sinus. For the detection of engraftment and chimerism, blood was collected from the tail of recipient mice at 2 week, 3, 4, 6, 7 and 9 months after transplantation for at least 4 months after transplantation. To observation of donor cells in peripheral blood, red blood cells were lysed with ammonium potassium buffer and the residual cells were stained with specific antibodies against CD45.1 (PE anti-mouse CD45.1 Antibody) and CD45.2 (APC anti-mouse CD45.2 Antibody).

3.2.6 Preparation Of The Tissue Samples From Transgenic Mice And Alkaline Lysis DNA Extraction For Genotyping

For alkaline lysis procedure, the mice tissue was collected from the tail or ear of mice. The received tissue was put into the 1,5 ml eppendorf tube. Optimizations of the alkaline lysis DNA extraction method were based on minimum time but maximum efficiency should be. Alkaline lysis DNA extraction method was applied. As an alkaline solution 200 µl 1mM ethylenediaminetetraacetic acid disodium salt dihydrate

(Na2EDTA), 25mM NaOH, pH: 12) was used and the sample was incubated at 98°C for 11 minutes inside the alkaline solution. When the incubation time was over, neutralization solution were added inside the alkaline solution (40mM Trizma® HCl, pH: 7.5) containing the sample in order to stop the lysis reaction. The resulting DNA samples should be kept at -20°C.

3.2.7 PCR genotyping

Traditional PCR was used to detection of GFP/+ mice or wild type mice, the polymerase enzyme only amplifies the target sequence. Three different primer sets were used in traditional PCR in order to choose the most suitable primer size. The couple of primers were used to genotype a-catulinGFP allele: Cin-G1, 59-GAAGTAGTGGCACAAGGGTAGGGG-39; Cin-G2, 59 GGCCGCGGTACCTGAGAAAC-39; Cin-G3, 59-GTTGCCGTCGTCCTTG AAGAAG-39. Traditional PCR was set up with T100 thermal cycler. The Taq 2X Master was used as a DNA polymerase enzyme master mix. Conditions for traditional PCR were as follow; 95°C for 2 minutes, 37 cycles of PCR including; denaturation at 95°C for 2 minutes, annealing at 60°C for 20 seconds and extension at 72°C for 1 minute with the final extension of 72°C for 5 minutes. Final concentrations of the out primers were set to 10 uM in dH2O. DMSO was used at a 5% final concentration as a fidelity enhancer.

All PCR samples were observed via agarose gel electrophoresis inside tris/borate/EDTA (TBE). TBE was prepared by mixing 10.8% trizma® base, 5.5% boric acid and 25% 0.5M Na2EDTA pH: 8.0 in dH2O. Agarose gel concentrations were calculated according to product sizes such as; 2 percent agarose concentration was used for products between 50-500bp. In order to sink the DNA content, final concentration of 1X loading dye and to estimate the product sizes, Gene Ruler 1 kb Plus DNA Ladder was used. For all visualization procedures, UV imaging system is used. For UV based visualization, 5 percent final concentration of ethidium bromide (EtBr) was added during preparation of the agarose gel. If not specifically mentioned, all PCR master mix ingredients were settled according to the manufacturer's protocol of the polymerase

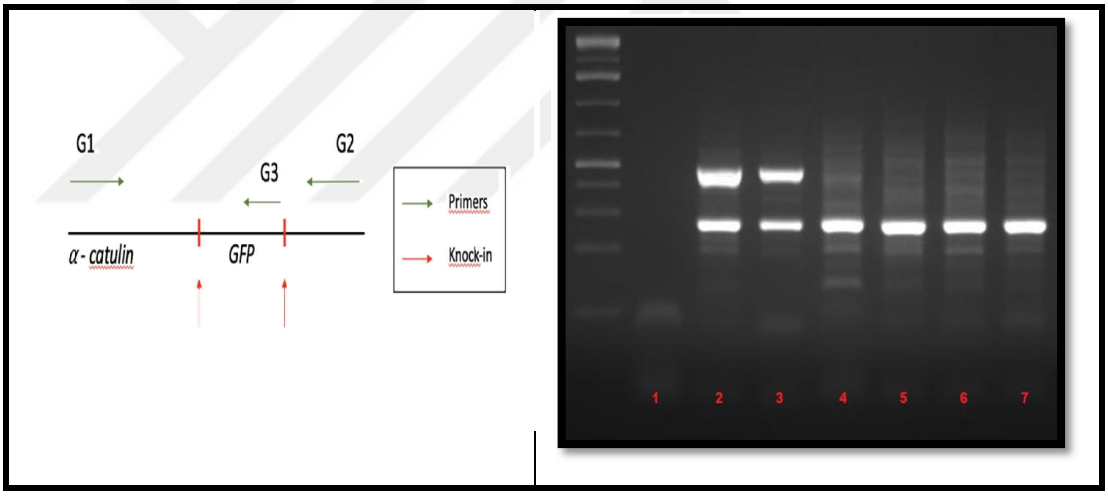
enzyme or its master mix.

4. RESULTS AND DISCUSSION

4.1 DETECTION OF GFP/WT MICE

Based on previous knowledge about PCR and a-catulin protein, we tested our designed primers for GFP/WT and WT/WT for PCR analysis by use of our optimizations. Negative control was used in order to detect the risk of contamination.

Figure 4.1: Detection of a-catulin proteins in transgenic mice one gel electrophoresis results of PCR based amplification via our specific primers.



Reference: This figure was prepared by Deniz ALTUNSU

1: Negative control

2-3: GFP/WT Samples

4-7: WT/WT Samples

4.2 OPTIMIZATION OF RADIATION THERAPHY

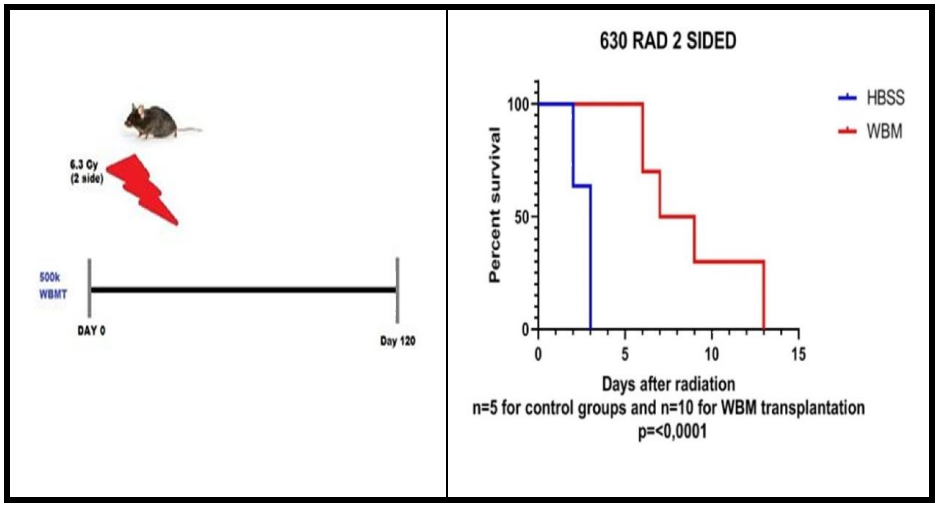
Bone marrow, spleen, fetal system and also spine bone have the most important

functions in transplantation experiments. First, bone marrow acts as a reconstitution mechanism in all mammals and recoveries cells from oxidative caused of radiation stress. Secondly, bm and spleen cells can modulate and stimulate diverse cellular processes. BMT and ST is an excellent candidate for reconstitution of hematopoiesis after radiation therapy. BMT and ST preserve C57BL/6 mice against radiation-induced hematological damage and death when given immediately after radiation exposure. To test whether the recovery effect of BMT and spleen transplantation can be applied to irradiated transgenic mice. If BMT and ST are effective when given at least 1 h after irradiation, C57BL mice were irradiated total body with different radiation dose. After radiation, the transgenic mice were given retro-orbital venous sinus HBSS control buffer or bone marrow and spleen cells at 500.000 and 1 million per mouse. The mouse survival was observed for at least 6 months.

4.2.1 After radiation, wbm transplantation

4.2.1.1 Transgenic mice were irradiated with 630 rad lethal dose at two sides

Figure 4.2: 630 rad treatment scheme for C57BL/6 mice and the recover effects of 500k WBMT.



Reference: This figure was prepared by Deniz ALTUNSU.

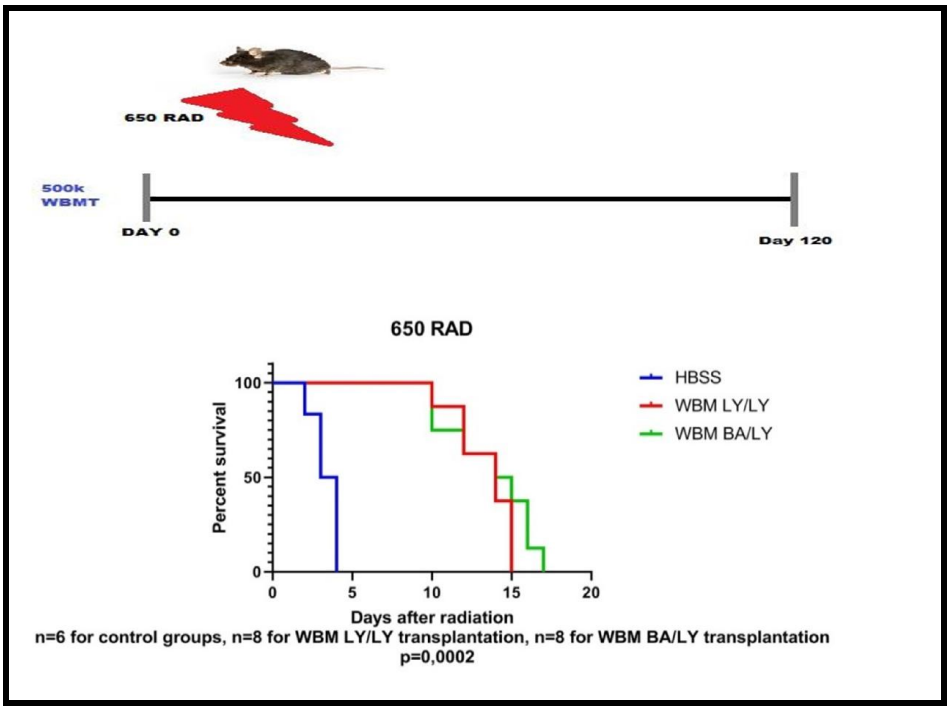
First of all, C57BL/6 mice were irradiated with 630 rad two sides, and 500k WBM cells

were given retro-orbital venous sinus immediately after irradiation. After radiation, the transgenic mice were given retro-orbital venous sinus HBSS control buffer or BM cells at 500.000 per mouse. To analyze the recovery effects of bone marrow cells, Kaplan-Meier survival curve depicts the 13 days survival. Just 5 male mice are used for saline-treated, lethally irradiated mice, and 10 male mice are used for BM cells-treated, lethally irradiated mice. Three days after radiation, all mice without BMT have died. After a few days, we observed both infection in mice and difficulty in walking the mice. We thought the nervous system was damaged by the radiation because of an overdose. According to ethical rules, these mice exposed to cervical dislocation.

We analyzed the recovery effects of WBM cells. Kaplan-Meier survival curve shows the 15 day survival (n = 5 for saline-treated, lethally irradiated mice, and n = 10 for WBM cell-treated, lethally irradiated mice).

4.2.1.2 Transgenic mice were irradiated with 650 rad lethal dose.

Figure 4.3: 650 rad treatment scheme for C57BL/6 mice and the recovery effects of 500k WBM cells.



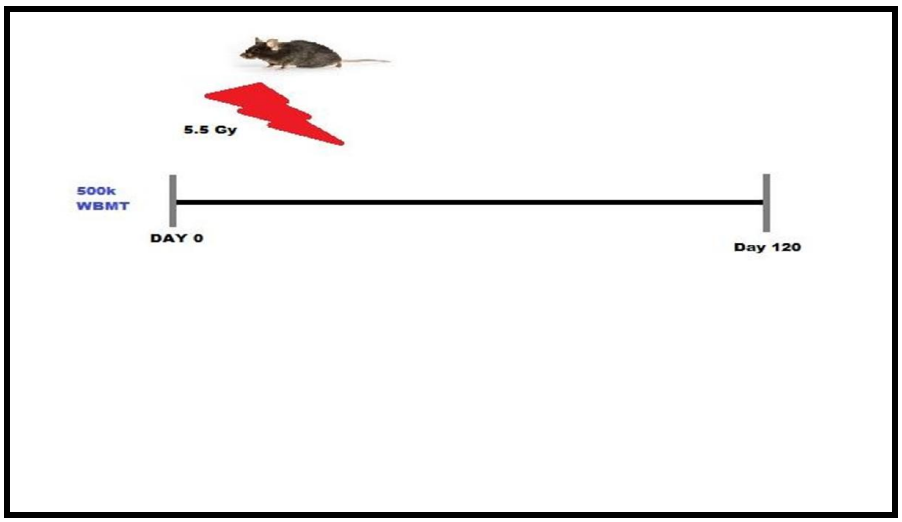
Reference: This figure was prepared by Deniz ALTUNSU.

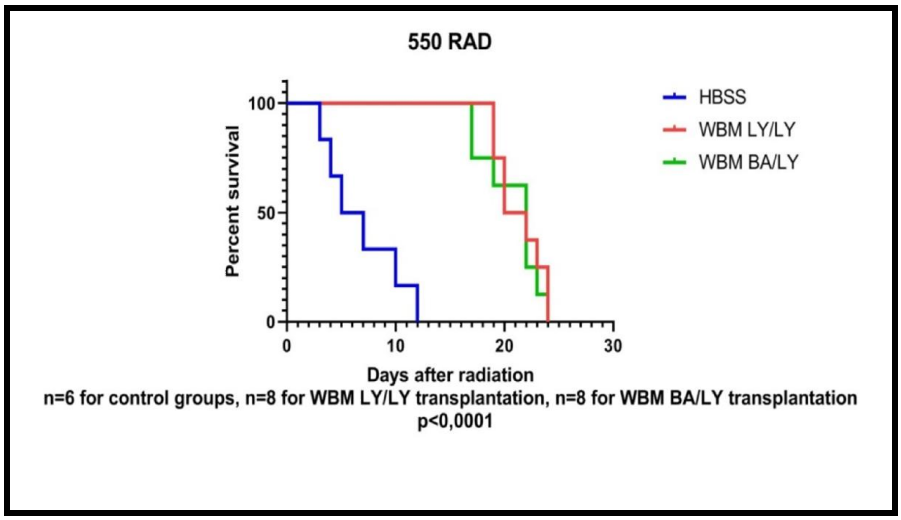
C57BL/6 mice were irradiated with 650 rad x 2, and 500k WBM cells were given retro-orbital venous sinus immediately after irradiation. After one side radiation dose of 650 rad, the transgenic mice were given retro-orbital venous sinus HBSS control buffer or BM cells from different population at 500.000 per mouse. To analyze the recovery effects of bone marrow cells, Kaplan-Meier survival curve depicts the 20 days survival. Just 6 male mice are used for saline-treated, lethally irradiated mice, and 16 male mice are used for BM cells-treated, lethally irradiated mice. After radiation therapy, all mice without BMT have died within five days. After a few days, we observed both infection in mice and difficulty in walking the mice. We thought the nervous system was damaged by the radiation because of an overdose. According to ethical rules, these mice exposed to cervical dislocation. This run was finished within 17-days.

We showed the recovery effects of WBM cells. Kaplan-Meier survival curve shows the 20 day survival (n = 6 for saline-treated, lethally irradiated mice, and n = 8 for BA/LY WBM and n=8 for LY/LY WBM cell-treated, lethally irradiated mice).

4.2.1.3 Transgenic mice were irradiated with 550 rad lethal dose

Figure 4.4: 550 rad treatment scheme for C57BL/6 mice and the recovery effects of 500k WBMT.





Reference: This figure was prepared by Deniz ALTUNSU

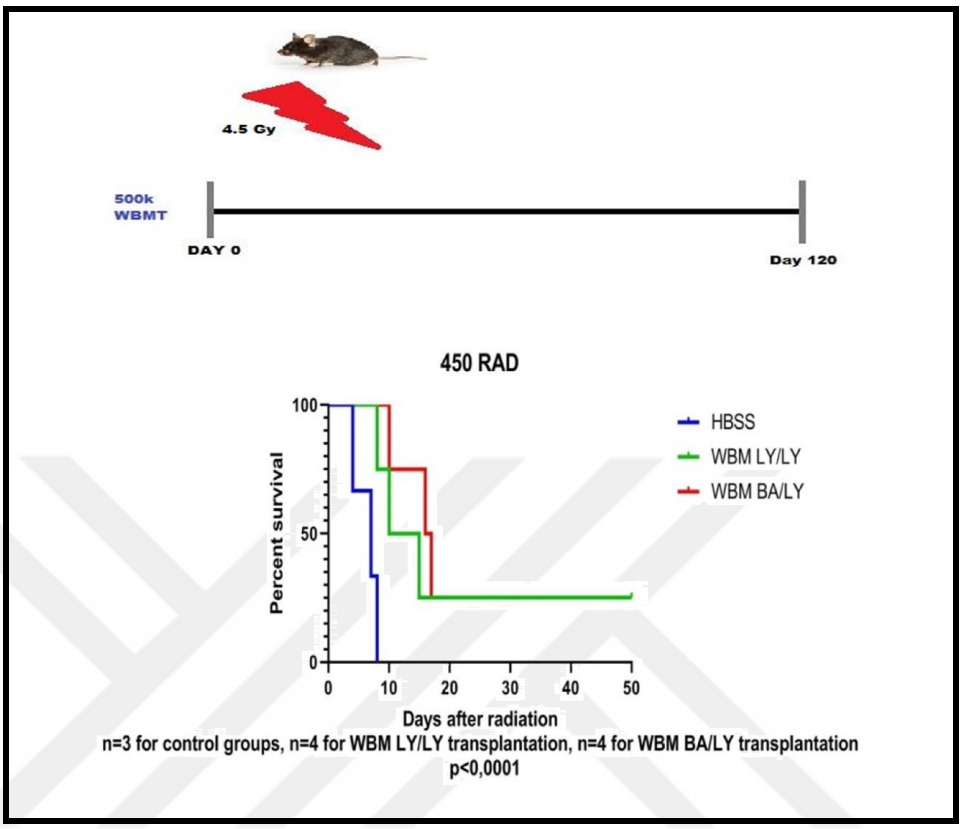
C57BL/6 mice were irradiated with 550 rad x 2, and 500k WBM cells were given retro-orbital venous sinus immediately after radiation. After two doses of 550 rad, the transgenic mice were given retro-orbital venous sinus HBSS control buffer or BM cells from different population at 500.000 per mouse. To analyze the recovery effects of bone marrow cells, Kaplan-Meier survival curve depicts the 30 days survival. Just 6 male mice are used for saline-treated, lethally irradiated mice, and 16 male mice are used for BM cells-treated, lethally irradiated mice. After radiation therapy, all mice without BMT has died within 12 days. After a few days, we observed both infection in mice and difficulty in walking the mice. We thought the nervous system was damaged by the radiation because of an overdose. According to ethical rules, these mice exposed to cervical dislocation. This run was finished within 25-days.

We analyzed the recovery effects of WBMT. Kaplan-Meier survival curve shows the 25 day survival (n = 8 for saline-treated, lethally irradiated mice, and n = 8 for BA/LY WBM and n=8 for LY/LY WBM cell-treated, lethally irradiated mice).

4.2.1.4 Transgenic mice were irradiated with 450 rad lethal dose

Figure 4.5: 450 rad treatment scheme for C57BL/6 mice and the recover

effects of 500k WBMT.



Reference: This figure was prepared by Deniz ALTUNSU.

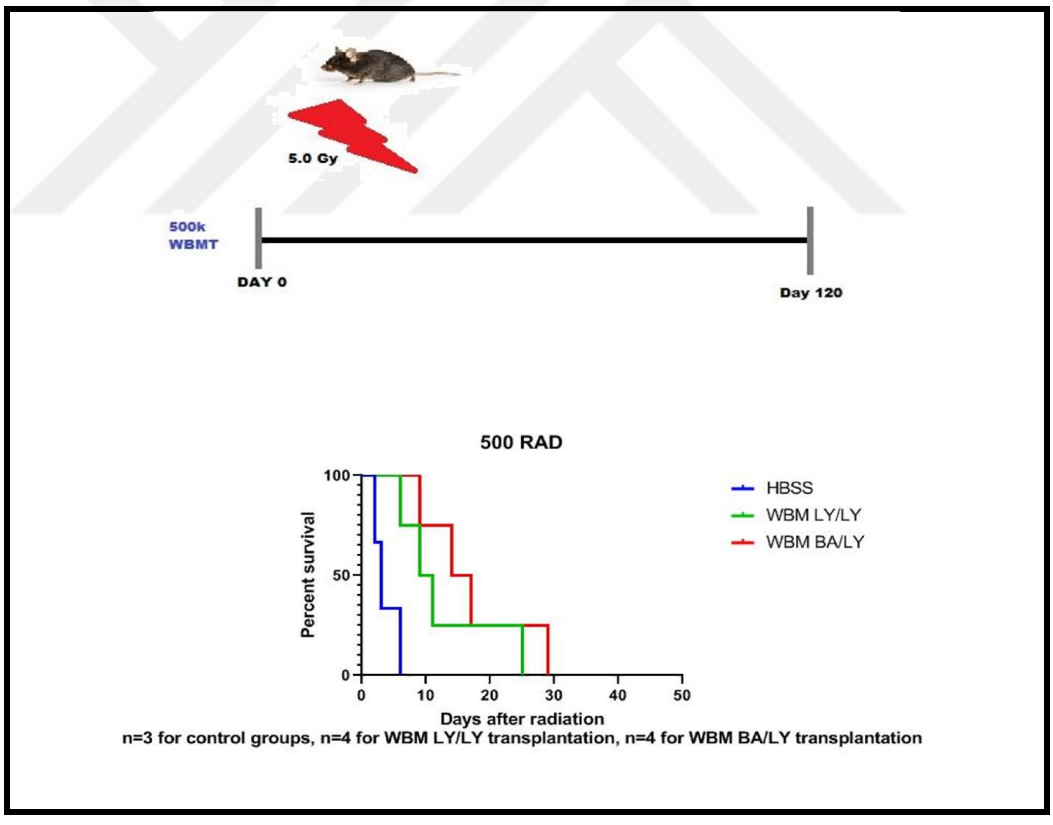
C57BL/6 mice were irradiated with two doses of 450 rad, and 500k WBM cells were given retro-orbital venous sinus immediately after irradiation. After radiation two dose of 450 rad, the transgenic mice were given retro-orbital venous sinus HBSS control buffer or BM cells from different population at 500.000 per mouse. To analyze the recovery effects of bone marrow cells, Kaplan-Meier survival curve depicts the 9 months survival. Just 3 male mice are used for saline-treated, lethally irradiated mice, and 8 male mice are used for BM cells-treated, lethally irradiated mice. After radiation therapy, all mice without BMT have died within 9- days. After a few days, we observed both infection in mice and difficulty in walking the mice. We thought the nervous system was damaged by the radiation because of an overdose. According to ethical rules, these mice exposed to cervical dislocation and some irradiated-mice died spontaneously. 25 percent of WBM-LY/LY and 25 percent of WBM-BA/LY treated-mice survived the radiation whereas all of saline-treated mice did not survive. These

results suggested that WBMT mitigates a lethal dose of TBI and reconstitute the hematopoietic system when administered immediately after radiation exposure.

Lastly, we showed that the recovery effects of WBMT. Kaplan-Meier survival curve depicts the nine months survival (n = 3 for saline-treated, lethally irradiated mice, and n = 4 for BA/LY WBM and n=4 for LY/LY WBM cell-treated, lethally irradiated mice).

4.2.1.5 Transgenic mice were irradiated with 500 rad lethal dose

Figure 4.6: 500 rad treatment scheme for C57BL/6 mice and the recovery effects of 500k WBMT.



Reference: This figure was prepared by Deniz ALTUNSU.

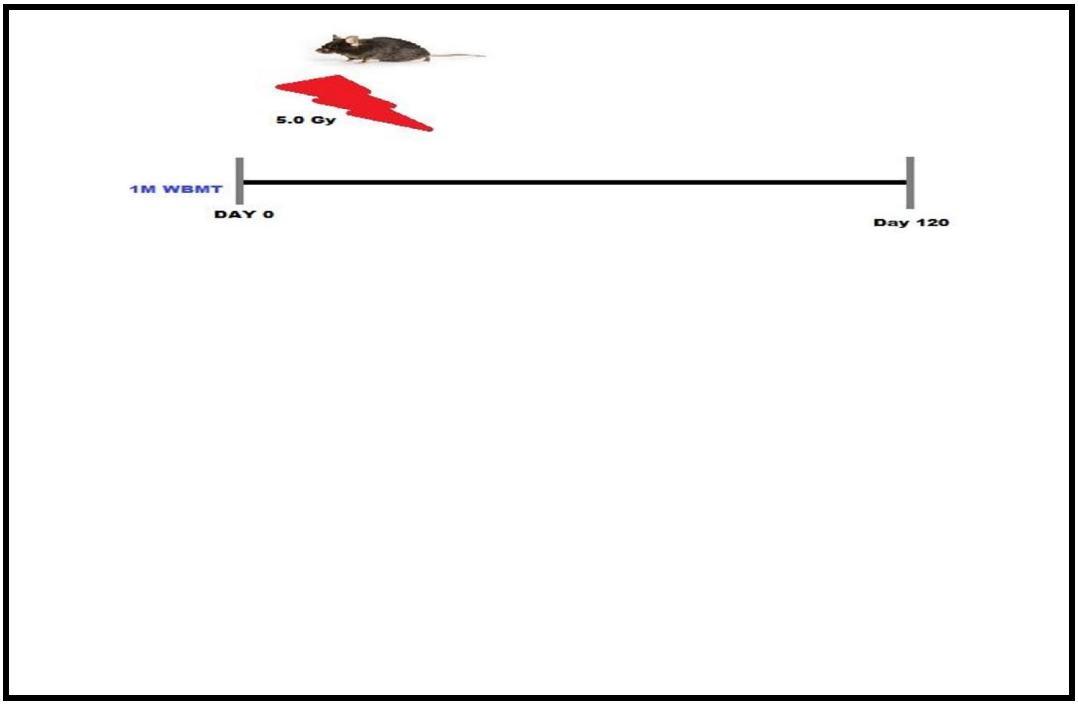
C57BL/6 mice were irradiated with 500 rad x 2, and 500k WBM cells were given retro-orbital venous sinus immediately after radiation. After radiation two dose of 500 rad,

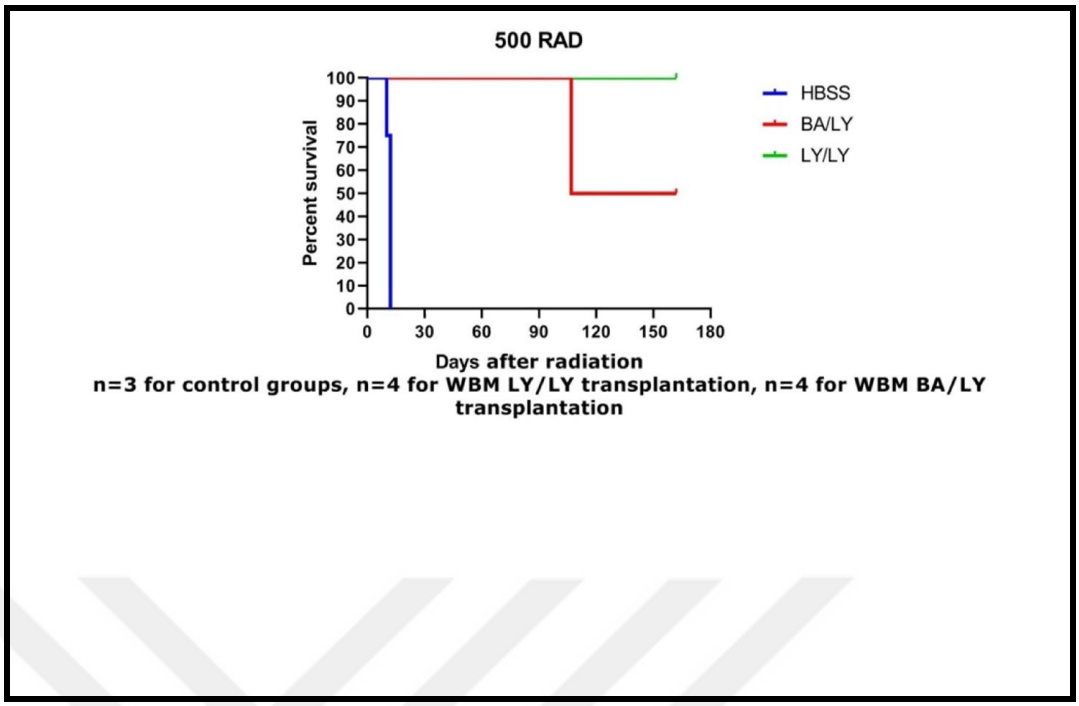
the transgenic mice were given retro-orbital venous sinus HBSS control buffer or BM cells from different population at 500.000 per mouse. To analyze the recovery effects of bone marrow cells, Kaplan-Meier survival curve depicts the 30 days survival. Just 3 male mice are used for saline-treated, lethally irradiated mice, and 8 male mice are used for BM cells-treated, lethally irradiated mice. After radiation therapy, all mice without BMT have died within 10 days. After a few days, we observed both infection in mice and difficulty in walking the mice. We thought the nervous system was damaged by the radiation because of an overdose. According to ethical rules, these mice exposed to cervical dislocation. This run was finished within 30-days.

We demonstrated that the recovery effects of WBMT. Kaplan-Meier survival curve depicts the 30 days survival (n = 3 for saline-treated, lethally irradiated mice, and n = 4 for BA/LY WBM and n=4 for LY/LY WBM cell-treated, lethally irradiated mice).

4.2.1.6 Transgenic mice were irradiated with 500 and 525 rad lethal dose with bolus

Figure 4.7: 500 rad treatment with bolus scheme for C57BL/6 mice and the recovery effects of 1m WBMT.



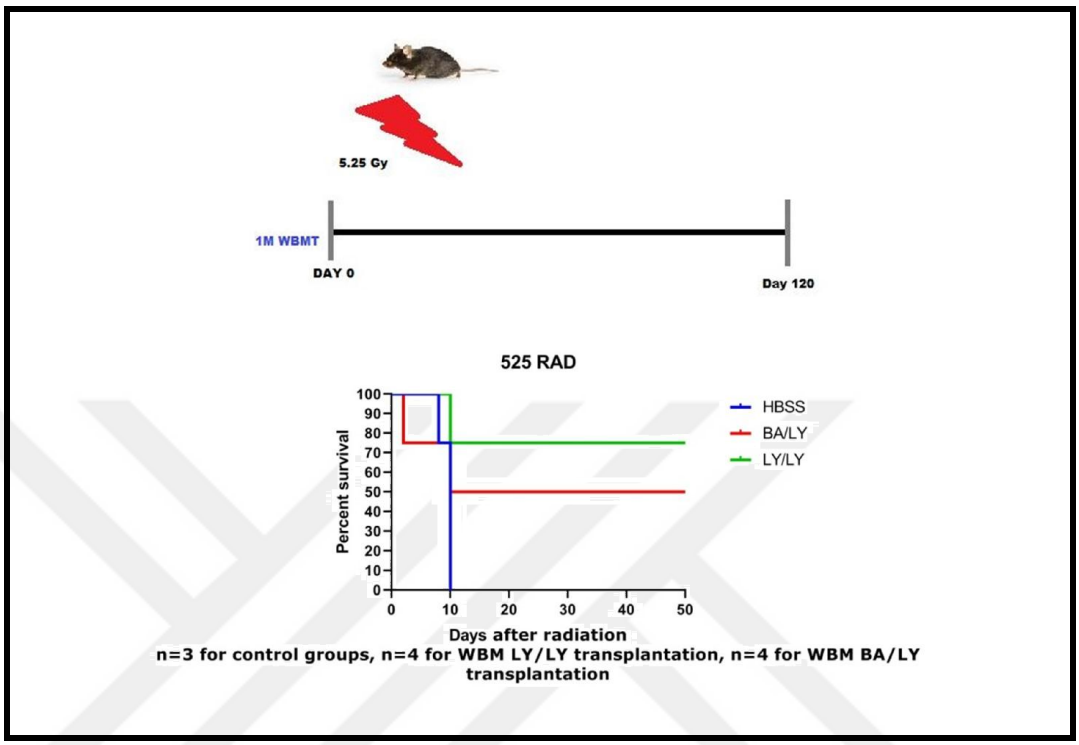


Reference: This figure was prepared by Deniz ALTUNSU.

C57BL/6 mice were irradiated with 500 rad x 2, and 1million WBM cells were given retro-orbital venous sinus immediately after irradiation. After radiation two dose of 500 rad, the transgenic mice were given retro-orbital venous sinus HBSS control buffer or BM cells from different population at 1 million per mouse. During the radiation experiment, bolus is used for reduce or alter dosing for targeted radiation therapy. The bolus is a matter which has features equal to tissue while irradiated. To analyze the recovery effects of bone marrow cells, Kaplan-Meier survival curve depicts the 6 months survival. Just 3 male mice are used for saline-treated, lethally irradiated mice, and 8 male mice are used for BM cells from different populations-treated, lethally irradiated mice. After radiation therapy, all mice without BMT have died within 2 weeks as we thought. After radiation therapy, we did not observe any infection in mice and difficulty in walking the mice. We assumed that radiation dose is not an overdose for mice. 100 percent of WBM-LY/LY and 50 percent of WBM-BA/LY treated-mice survived the radiation whereas all of saline-treated mice did not survive. These results suggested that WBMT mitigates a lethal dose of TBI and reconstitute the hematopoietic system when administered immediately after radiation exposure.

We showed that the recovery effects of WBMT. Kaplan-Meier survival curve depicts the 6 months survival (n = 3 for saline-treated, lethally irradiated mice, and n = 4 for BA/LY WBM and n=4 for LY/LY WBM cell-treated, lethally irradiated mice).

Figure 4.8: 525 rad treatment with bolus scheme for C57BL/6 mice and the recovery effects of 1m WBM cells.



Reference: This figure was prepared by Deniz ALTUNSU.

C57BL/6 mice were irradiated with 525 rad x 2, and 1million WBM cells were given retro-orbital venous sinus immediately after irradiation. After radiation two dose of 525 rad, the transgenic mice were given retro-orbital venous sinus HBSS control buffer or BM cells from different population at 1 million per mouse. During the radiation experiment, bolus is used for reduce or alter dosing for targeted radiation therapy. The bolus is a matter which has features equal to tissue while irradiated. To analyze the recovery effects of bone marrow cells, Kaplan-Meier survival curve depicts the 6 months survival. Just 3 male mice are used for saline-treated, lethally irradiated mice, and 8 male mice are used for BM cells from different populations-treated, lethally irradiated mice. After radiation therapy, all mice without BMT have died within 10-days as we thought. After radiation therapy, we did not observe any infection in mice and difficulty in walking the mice. We thought that radiation dose is not an overdose for mice. 75 percent of WBM-LY/LY and 50 percent of WBM-BA/LY treated-mice

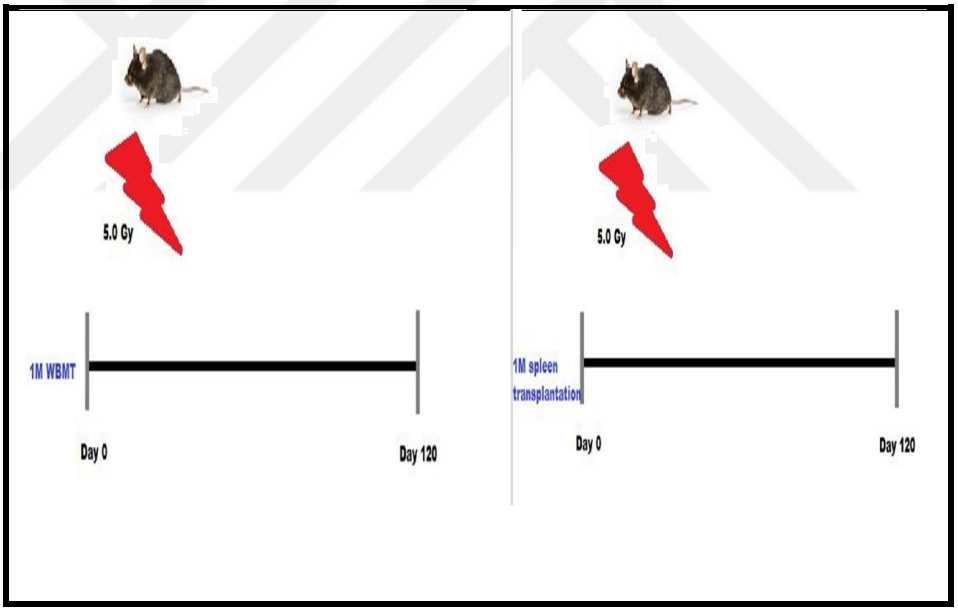
survived the radiation whereas all of saline-treated mice did not survive. These results suggested that WBMT mitigates a lethal dose of TBI and reconstitute the hematopoietic system when administered immediately after radiation exposure.

We analyzed that recovery effect of WBMT. Kaplan-Meier survival curve depicts the 6 months survival (n = 3 for saline-treated, lethally irradiated mice, and n = 4 for BA/LY WBM and n=4 for LY/LY WBM cell-treated, lethally irradiated mice).

4.2.2 After radiation, spleen and wbm transplantation

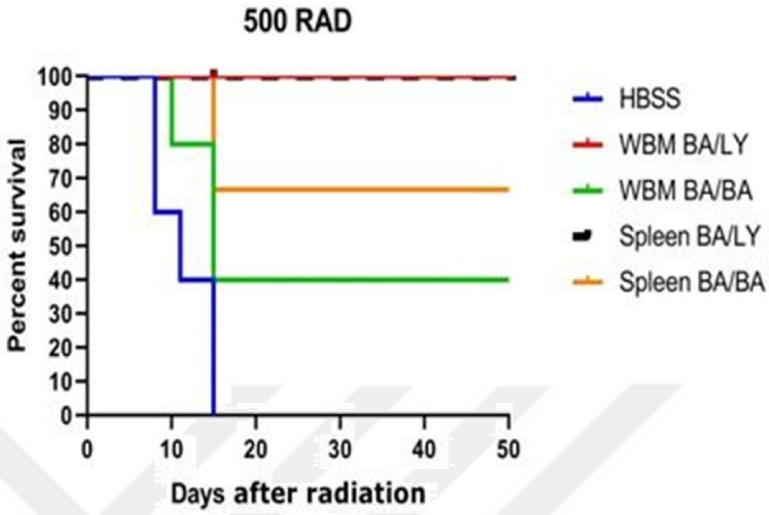
4.2.2.1 Transgenic mice were irradiated with 500 rad lethal dose with bolus, transplanted with spleen and wbm cells

Figure 4.9: 500 rad treatments with bolus scheme for C57BL/6 mice.



Reference: This figure was prepared by Deniz ALTUNSU

Figure 4.10: 500 rad treatments with bolus scheme for C57BL/6 mice.



n = 3 for saline-treated, lethally irradiated mice, and n = 4 for BA/LY WBM and spleen cells. In addition, n=4 for BA/BA WBM and spleen cell-treated, lethally irradiated mice

Reference: This figure was prepared by Deniz ALTUNSU

C57BL/6 mice were irradiated with 500 rad x 2. After radiation two dose of 500 rad, the transgenic mice were given retro-orbital venous sinus HBSS control buffer or BM cells from different population and spleen cells from different populations at 1 million per mouse. During the radiation experiment, bolus is used again for reduce or alter dosing for targeted radiation therapy. The bolus is a matter which has features equal to tissue while irradiated. To analyze the recovery effects of bone marrow cells, Kaplan-Meier survival curve depicts the 3 months survival. Just 6 male mice are used for saline-treated, lethally irradiated mice, and 8 male mice are used for BM cells from different populations-treated, lethally irradiated mice. After radiation therapy, all mice without BMT have died within 14-days as we thought. After radiation therapy, we did not observe any infection in mice and difficulty in walking the mice. We thought that radiation dose is not an overdose for mice. 100 percent of WBM-BA/LY and spleen BA/LY and 50 percent of WBM-BA/BA treated-mice survived the radiation whereas all of saline-treated mice did not survive. These results suggested that WBMT mitigates a

lethal dose of TBI and reconstitute the hematopoietic system when administered immediately after radiation exposure.

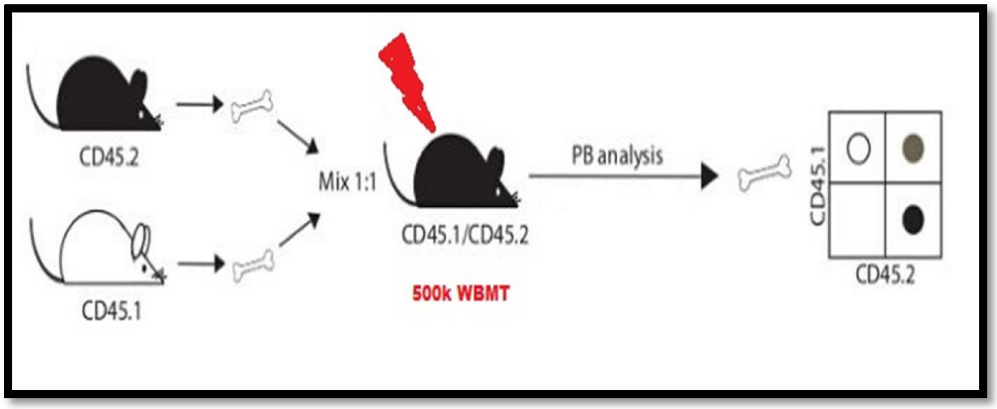
We showed that the recovery effects of WBM and ST. Kaplan-Meier survival curve depicts the 3 months survival (n = 3 for saline-treated, lethally irradiated mice, and n = 4 for BA/LY WBM and spleen cells. In addition, n=4 for BA/BA WBM and spleen cell-treated, lethally irradiated mice).

4.3 COMPETITIVE RECONSTITUTION ASSAYS IN IRRADIATED MICE

Competitive bone marrow transplantation is used for measuring multi-lineage reconstitution of hematopoiesis in irradiated transplant recipient mice.

In this experiment, hematopoietic stem and progenitor cells are determined functionality in vivo. The main idea is to transplant bone marrow donor cells (derived from transgenic mice of choice) on C57BL6 background together with normal competitor bone marrow. In order to separate the donor from competitor cells in transplantation, usually, competitor mice are congenic and carry the differential B cell antigen originally assigned Ly5.1 and CD45.1.

Figure 4.11: C57BL6 donor mice (CD45.2) and C57BL6 competitor mice (CD45.1).



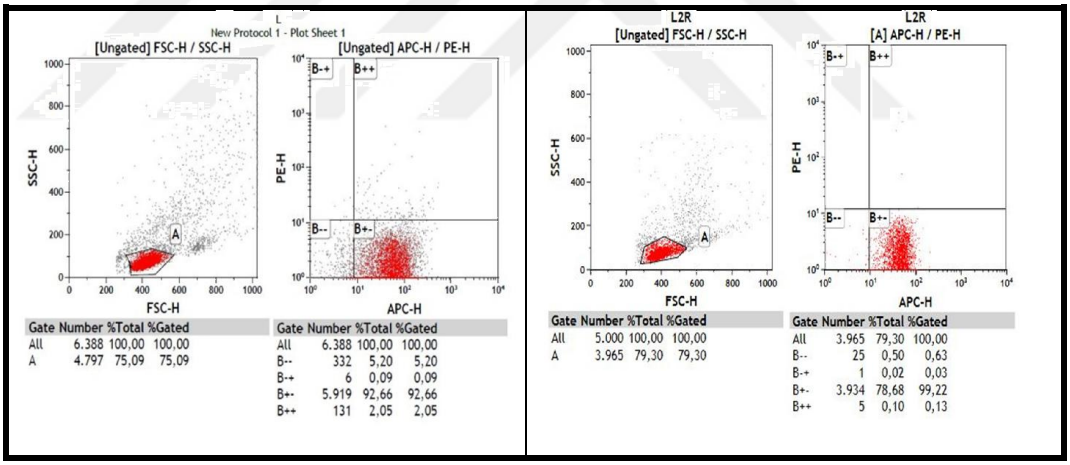
Reference: This figure was created by Deniz ALTUNSU

A standard competitive bone marrow transplantation experiment includes two transplantation groups, the donor (transgenic mice of choice and their controls) are transplanted in competition with normal competitors and engraftment performance is decided in both blood and bone marrow.

4.3.1. After 450 rad, ba/ba and ba/ly 500k wbm cells transplantation

These BA/BA (CD45.1) competitor mice were irradiated with 450 rad lethal dose. For a competitive transplant, bone marrow cells from donor mice (CD45.2 C57Bl6 background) retro-orbital injected into the irradiated recipient mice (CD45.1). PB was collected from tail of mice for the determination of the efficacy of bone marrow reconstitution. Peripheral blood (PB) was analyzed at 17-days, 7 and 9 months post-transplantation.

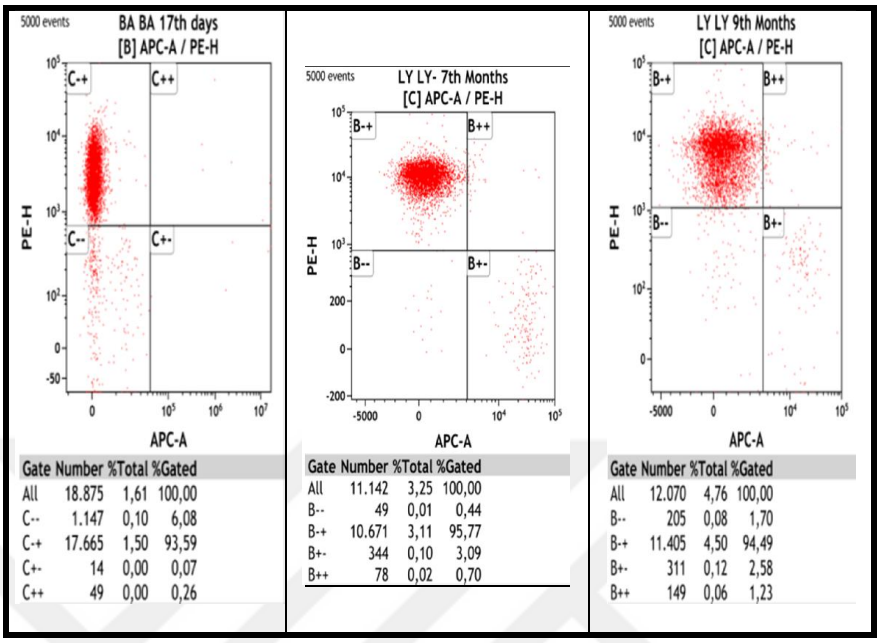
Figure 4.11: Peripheral blood mononuclear cells were gated on the basis of FSC vs. SSC profiles, excluding debris and nonlysed RBCs. These gated leukocytes were then probed for expression of either the CD45.1 or CD45.2 allele.



Reference: This figure was created by Deniz ALTUNSU.

The CD45.1 background host mice in which CD45.2 bone marrow cells have been transplanted show a chimeric-blood phenotype, however, at 17 days, 7th months and 9th months post-transplant.

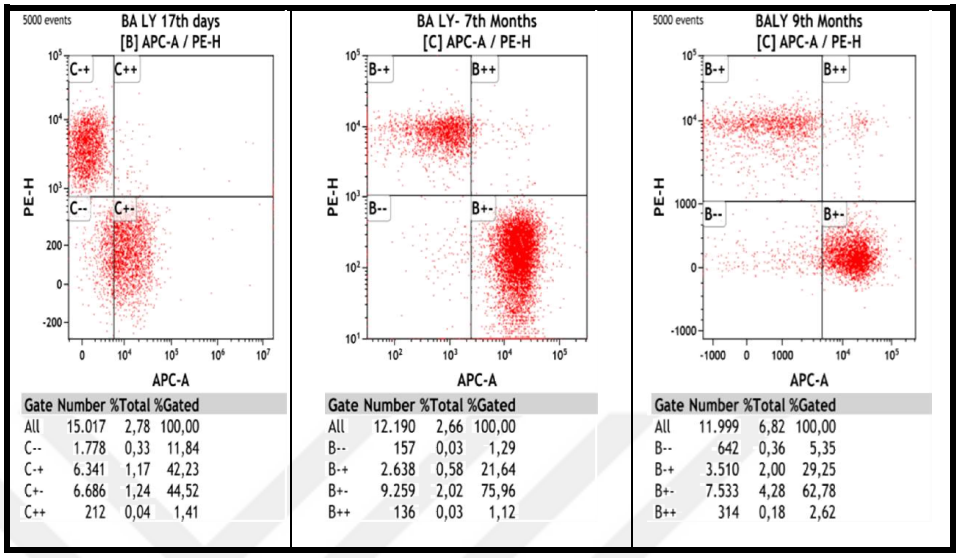
Figure 4.12: Transplantation with ly/ly transgenic mice.



Reference: This figure was created by Deniz ALTUNSU.

Peripheral blood (PB) was analyzed at 17-days, 7 and 9 months post-transplantation. According to the flow cytometer plots, donor ($CD45.2^+$) and competitor cells ($CD45.1^+$) is shifted for each lineage as shown. According to these results, 17 days after transplantation, donor cells are 93, 59 percent. At 7th month 95, 77 percent of donor cells were settled in recipient bone marrow. 94.49 percent of donor cells were settled in recipient BM at 9th month.

Figure 4.13: Transplantation with ba/ly transgenic mice.



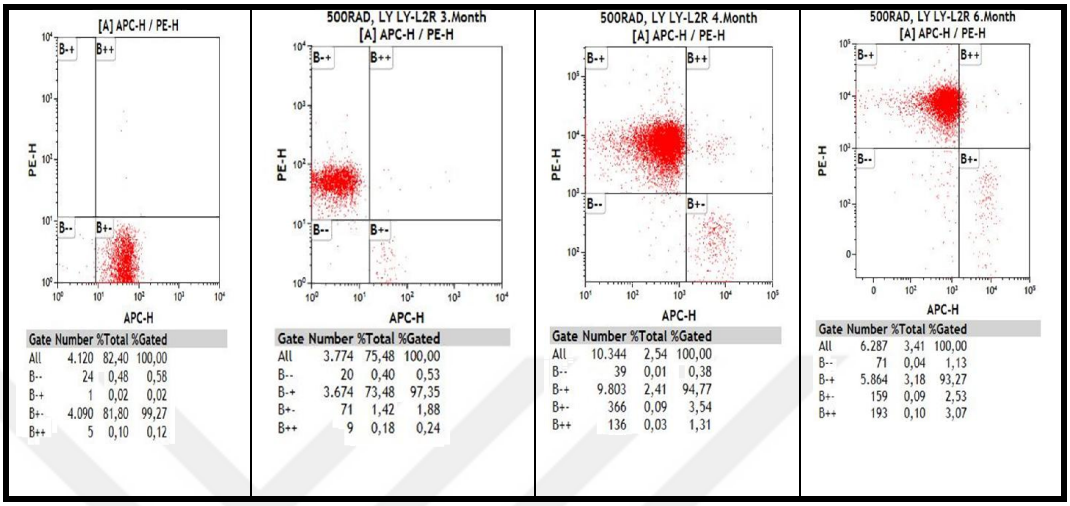
Reference: This figure was created by Deniz ALTUNSU.

These BA/BA (CD45.1) competitor mice were irradiated with 450 rad lethal dose. For a competitive transplant, 1:1 ratio of BM CD45.2:CD45.1 leukocytes, as they were transplanted into the mouse originally. Bone marrow cells from donor mice retro-orbital injected into the irradiated recipient mice (CD45.1). PB was collected from tail of mice for the determination of the efficacy of bone marrow reconstitution. Peripheral blood (PB) was analyzed at 17-days, 7 and 9 months post-transplantation. The CD45.1 background host mice in which CD45.2 bone marrow cells have been transplanted show a chimeric-blood phenotype, however, at 17 day, 7th month and 9th month post-transplant.

According to this results, 17 days after transplantation, chimeric-blood cells are 42, 23 percent LY/LY and 44, 52 percent BA/BA. At 7th month 21, 64 percent LY/LY and 75, 96 percent BA/BA of chimeric-blood cells were settled in recipient bone marrow. 29, 25 percent LY/LY and 62, 78 percent BA/BA of chimeric-blood cells were settled in recipient BM at 9th month.

4.3.2 After 500 rad, ly/ly and ba/ly 1m wbm cell transplantation

Figures 4.14: After 500 rad, transplantation with 1m ly/ly transgenic mice.

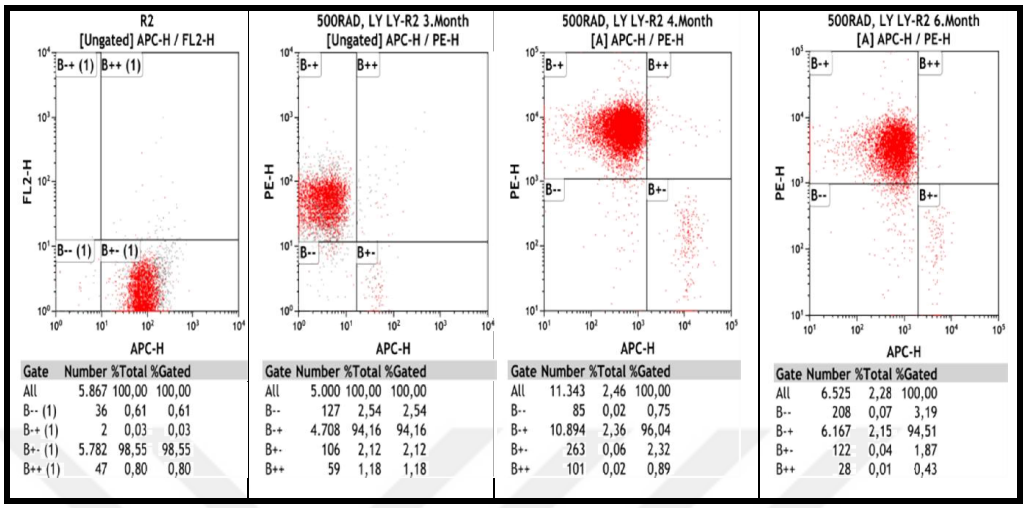


Reference: This figure was created by Deniz ALTUNSU.

These BA/BA (CD45.1) competitor mice were irradiated with 500 rad lethal dose. For a competitive transplant, BM cells from the CD45.2 were transplanted into the mouse originally. Bone marrow cells from donor mice were retro-orbital injected into the irradiated recipient mice (CD45.1). PB was collected from tail of mice for the determination of the efficacy of bone marrow reconstitution. Peripheral blood (PB) was analyzed at 3, 4 and 6 month post-transplantation. The CD45.1 background host mice in which CD45.2 bone marrow cells have been transplanted show a chimeric-blood phenotype, 3, 4 and 6 month post-transplantation.

According to the flow cytometer plots, donor ($CD45.2^{+}$) and competitor cells ($CD45.1^{+}$) is shifted for each lineage as shown. Three month after transplantation, chimeric-blood cells are 95, 32 percent LY/LY, at 4th month 94, 29 percent LY/LY and at 6th month 95, 72 percent LY/LY chimeric-blood cells were settled in recipient BM.

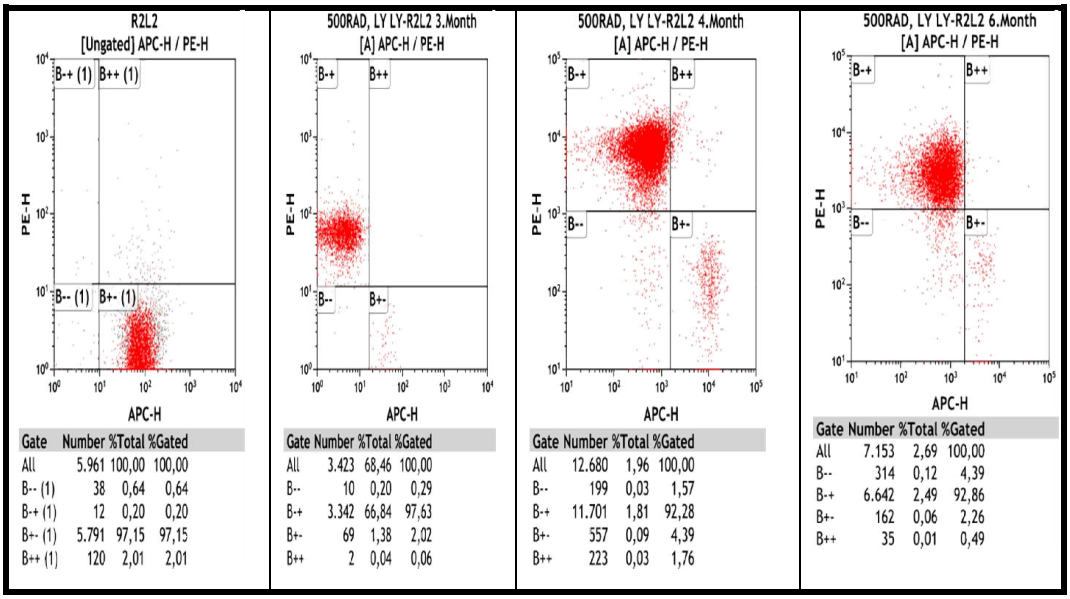
Figures 4.15: After 500 rad, transplantation with 1m ly/ly transgenic mice.



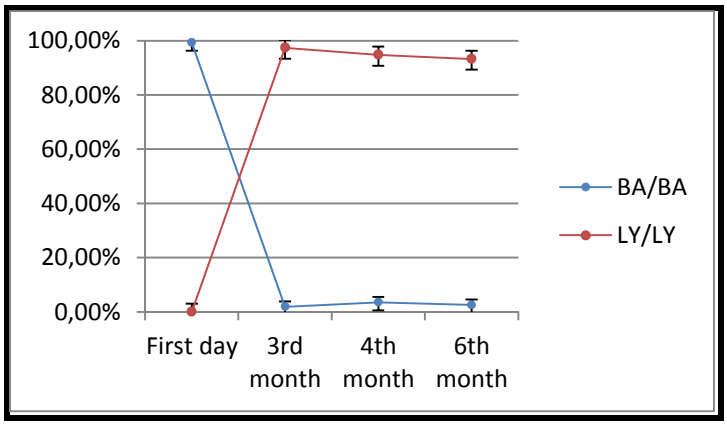
Reference: This figure was created by Deniz ALTUNSU.

According to this results, three month after transplantation, chimeric-blood cells are 94,16 percent LY/LY, at 4th month 96,04 percent LY/LY and at 6th month 94,51 percent LY/LY chimeric-blood cells were settled in recipient BM.

Figures 4.16: After 500 rad, transplantation with 1m ly/ly transgenic mice.



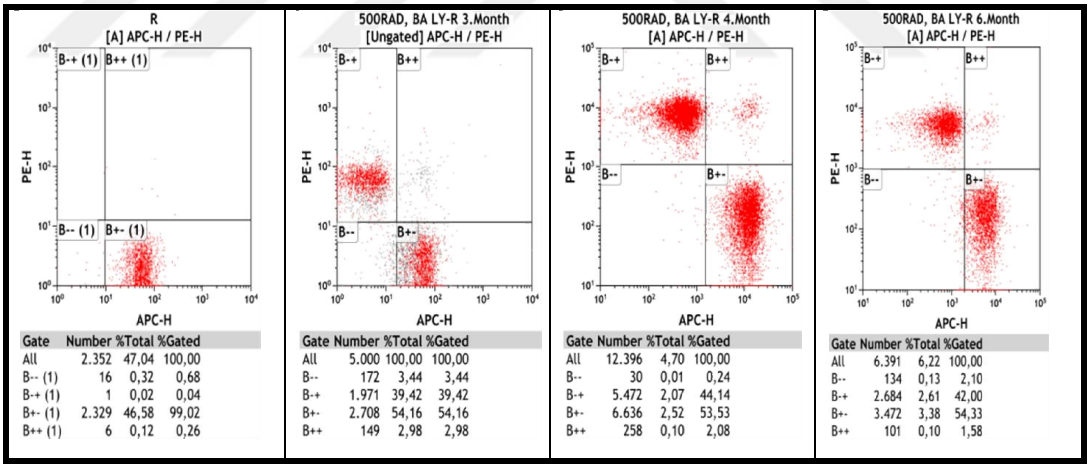
Reference: This figure was created by Deniz ALTUNSU.



Reference: This figure was created by Deniz ALTUNSU.

According to these results, three month after transplantation, chimeric-blood cells are 97,63 percent of LY/LY, at 4th month 92,28 percent of LY/LY and at 6th month 92,86 percent of LY/LY chimeric-blood cells were settled in recipient BM.

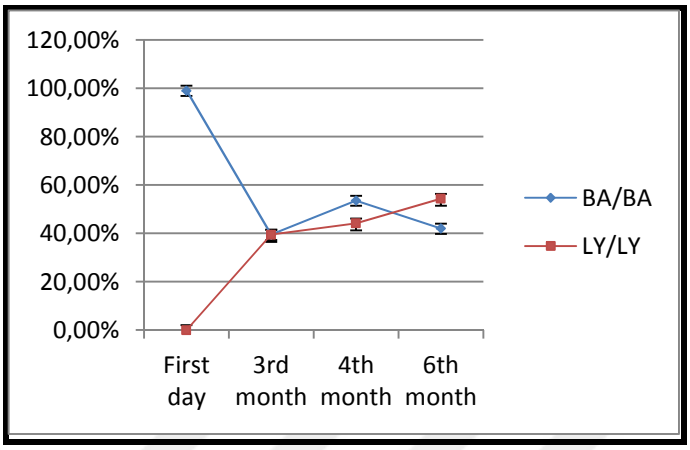
Figures 4.17: After 500 rad, transplantation with 1m ba/ly transgenic mice.



Reference: This figure was created by Deniz ALTUNSU.

These BA/BA (CD45.1) competitor mice were irradiated with 500 rad lethal dose. For a competitive transplant, 1:1 ratio of BM CD45.2:CD45.1 leukocytes, as they were transplanted into the mouse originally. Bone marrow cells from donor mice retro-orbital injected into the irradiated recipient mice (CD45.1). PB was collected from tail of mice for the determination of the efficacy of bone marrow reconstitution. Peripheral blood

(PB) was analyzed at 3, 4 and 6 month post-transplantation. The CD45.1 background host mice in which CD45.2/1 bone marrow cells have been transplanted show a chimeric-blood phenotype.

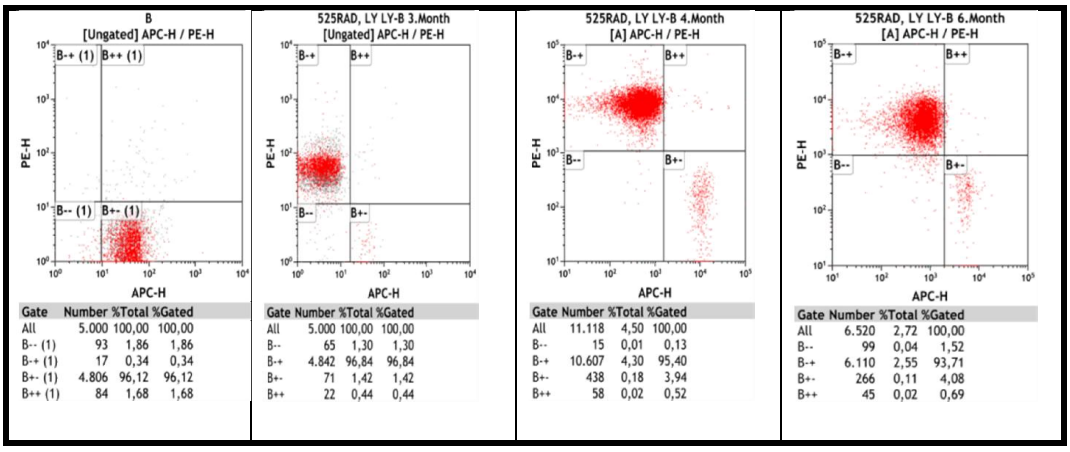


Reference: This figure was created by Deniz ALTUNSU.

According to this results, 3th month after transplantation, chimeric-blood cells are 39, 42 percent LY/LY and 54, 16 percent BA/BA. At 4th month 44, 14 percent LY/LY and 53, 53 percent BA/BA of chimeric-blood cells were settled in recipient bone marrow. 42, 00 percent LY/LY and 54, 33 percent BA/BA of chimeric-blood cells were settled in recipient BM at 6th month.

4.3.3 After 525 rad, ly/ly and ba/ly 1m wbm cell transplantation

Figure 4.18: After 525 rad, transplantation with 1m ly/ly transgenic mice.

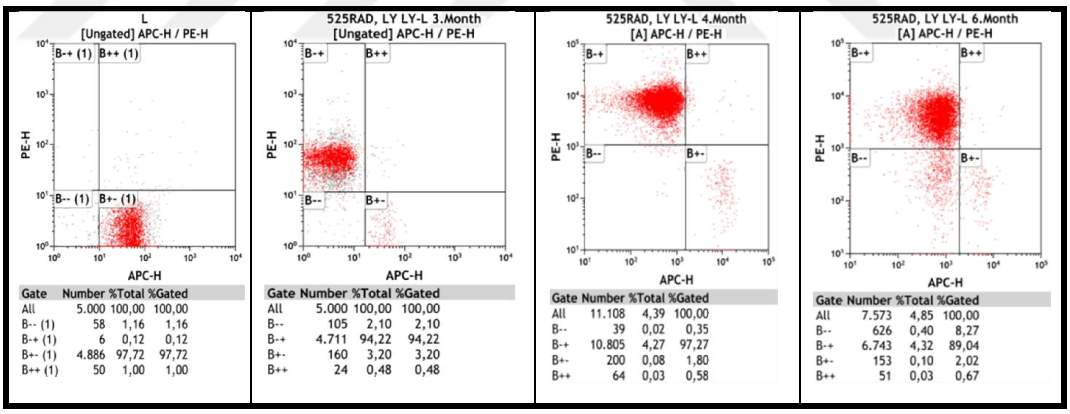


Reference: This figure was created by Deniz ALTUNSU.

These BA/BA (CD45.1) competitor mice were irradiated with 525 rad lethal dose. For a competitive transplant, BM cells from the CD45.2 were transplanted into the mouse originally. Bone marrow cells from donor mice retro-orbital injected into the irradiated recipient mice (CD45.1). PB was collected from tail of mice for the determination of the efficacy of bone marrow reconstitution. Peripheral blood (PB) was analyzed at 3, 4 and 6 month post-transplantation. The CD45.1 background host mice in which CD45.2 bone marrow cells have been transplanted show a chimeric-blood phenotype, however, at 3th, 4th and 6th month post-transplant.

According to this results, three month after transplantation, chimeric-blood cells are 96,84 percent LY/LY, at 4th month 95,40 percent LY/LY and at 6th month 93,71 percent LY/LY chimeric-blood cells were settled in recipient BM.

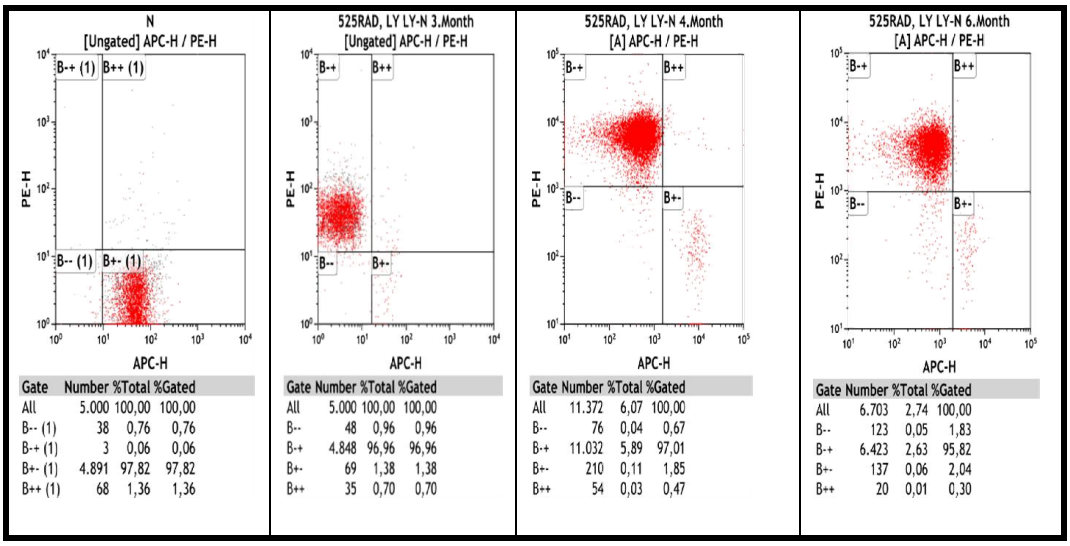
Figure 4.19: After 525 rad, transplantation with 1million LY/LY WBM cells



Reference: This figure was created by Deniz ALTUNSU.

According to this results, three month after transplantation, chimeric-blood cells are 94,22 percent LY/LY, at 4th month 97,27 percent LY/LY and at 6th month 89,04 percent LY/LY chimeric-blood cells were settled in recipient BM.

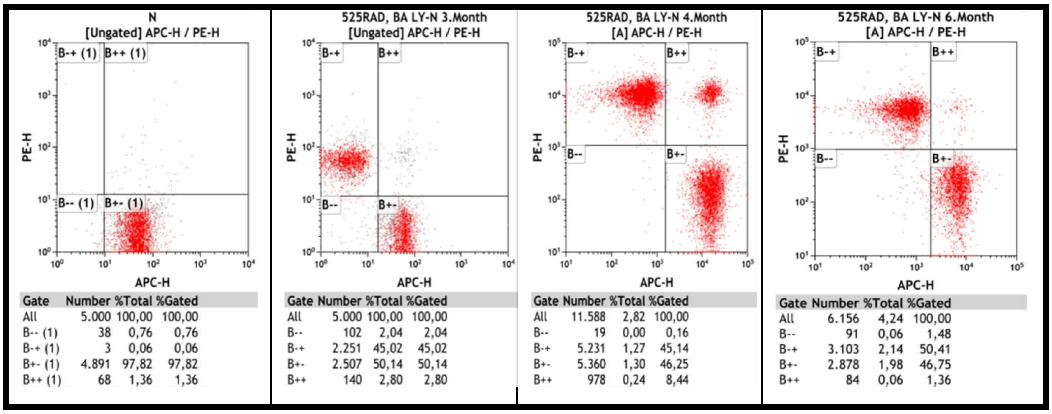
Figure 4.20: After 525 rad, transplantation with 1million LY/LY WBM cells



Reference: This figure was created by Deniz ALTUNSU.

According to this results, three month after transplantation, chimeric-blood cells are 96,96 percent LY/LY, at 4th month 97,01 percent LY/LY and at 6th month 95,82 percent LY/LY chimeric-blood cells were settled in recipient BM.

Figure 4.21: After 525 rad, transplantation with 1million BA/LY WBM cells



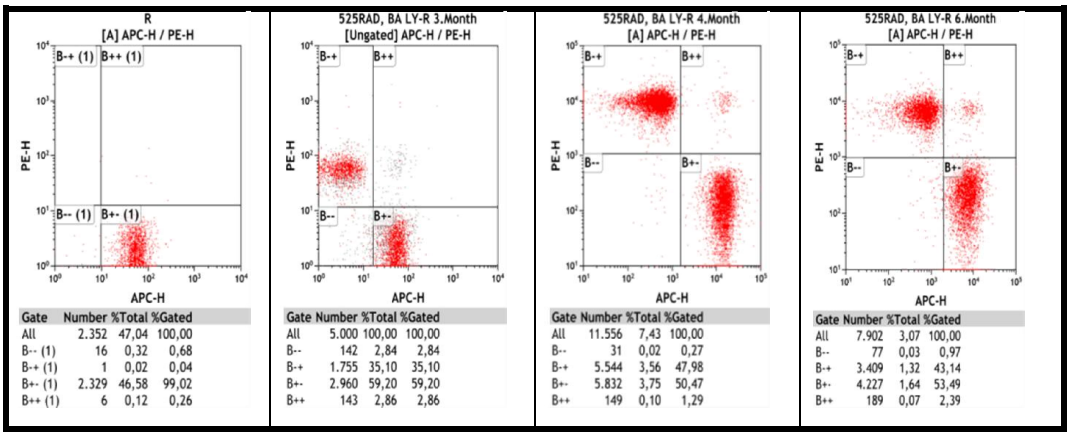
Reference: This figure was created by Deniz ALTUNSU.

These BA/BA (CD45.1) competitor mice were irradiated with 500 rad lethal dose. For a competitive transplant, 1:1 ratio of BM CD45.2:CD45.1 leukocytes, as they were transplanted into the mouse originally. Bone marrow cells from donor mice retro-orbital injected into the irradiated recipient mice (CD45.1). PB was collected from tail of mice

for the determination of the efficacy of bone marrow reconstitution. Peripheral blood (PB) was analyzed at 3, 4 and 6 month post-transplantation. The CD45.1 background host mice in which CD45.2 bone marrow cells have been transplanted show a chimeric-blood phenotype.

According to this results, 3th month after transplantation, chimeric-blood cells are 45, 02 percent LY/LY and 50, 14 percent BA/BA. At 4th month 45, 14 percent LY/LY and 46, 25 percent BA/BA of chimeric-blood cells were settled in recipient bone marrow. 50, 41 percent LY/LY and 46, 75 percent BA/BA percent of chimeric-blood cells were settled in recipient BM at 6th month.

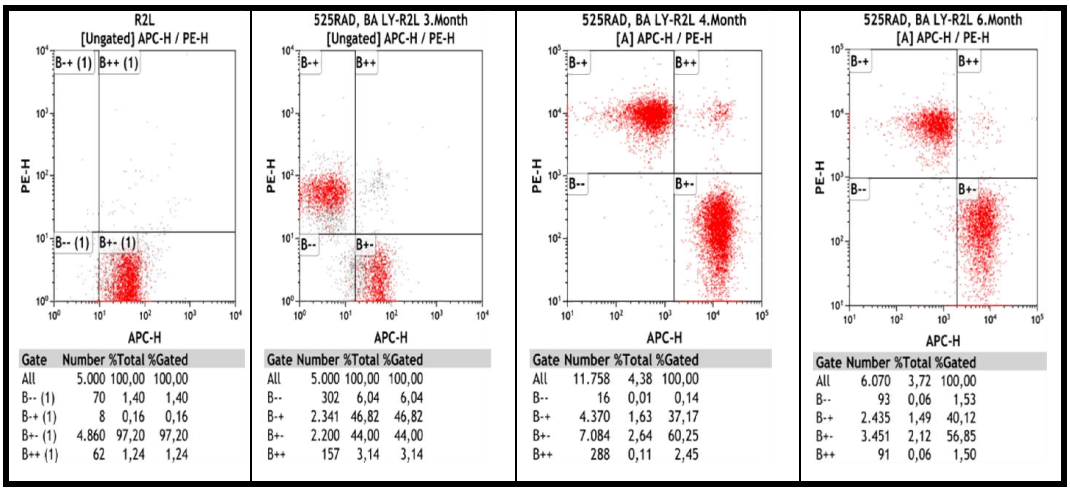
Figure 4.22: After 525 rad, transplantation with 1million BA/LY WBM cells.



Reference: This figure was created by Deniz ALTUNSU.

According to this results, 3th month after transplantation, chimeric-blood cells are 35, 10 percent LY/LY and 59, 20 percent BA/BA. At 4th month 47, 98 percent LY/LY and 50, 47 percent BA/BA of chimeric-blood cells were settled in recipient bone marrow. 43, 14 percent LY/LY and 53, 49 percent BA/BA percent of chimeric-blood cells were settled in recipient BM at 6th month.

Figure 4.23: After 525 rad, transplantation with 1million BA/LY WBM cells.



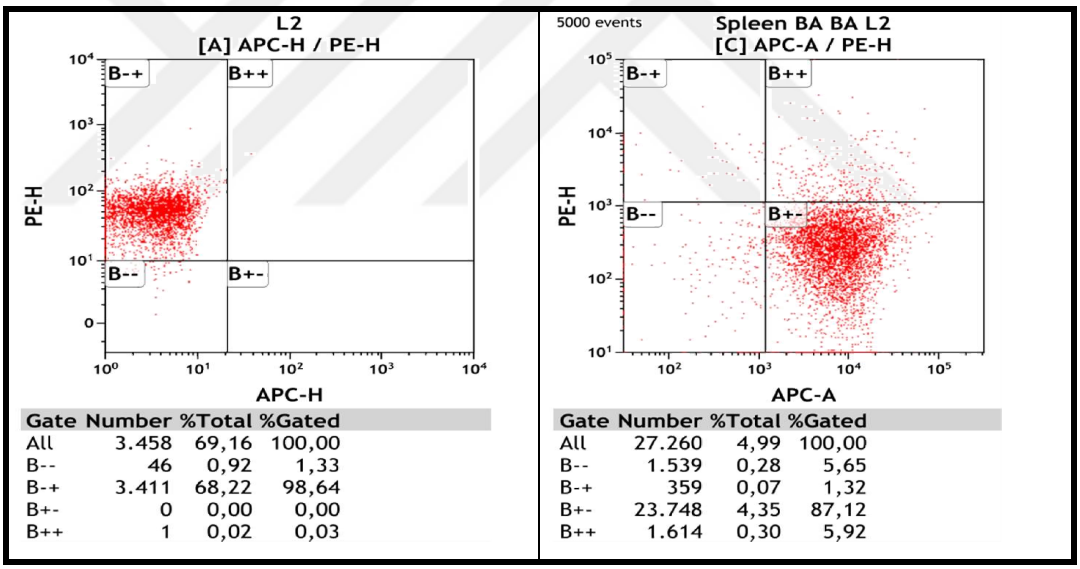
Reference: This figure was created by Deniz ALTUNSU.

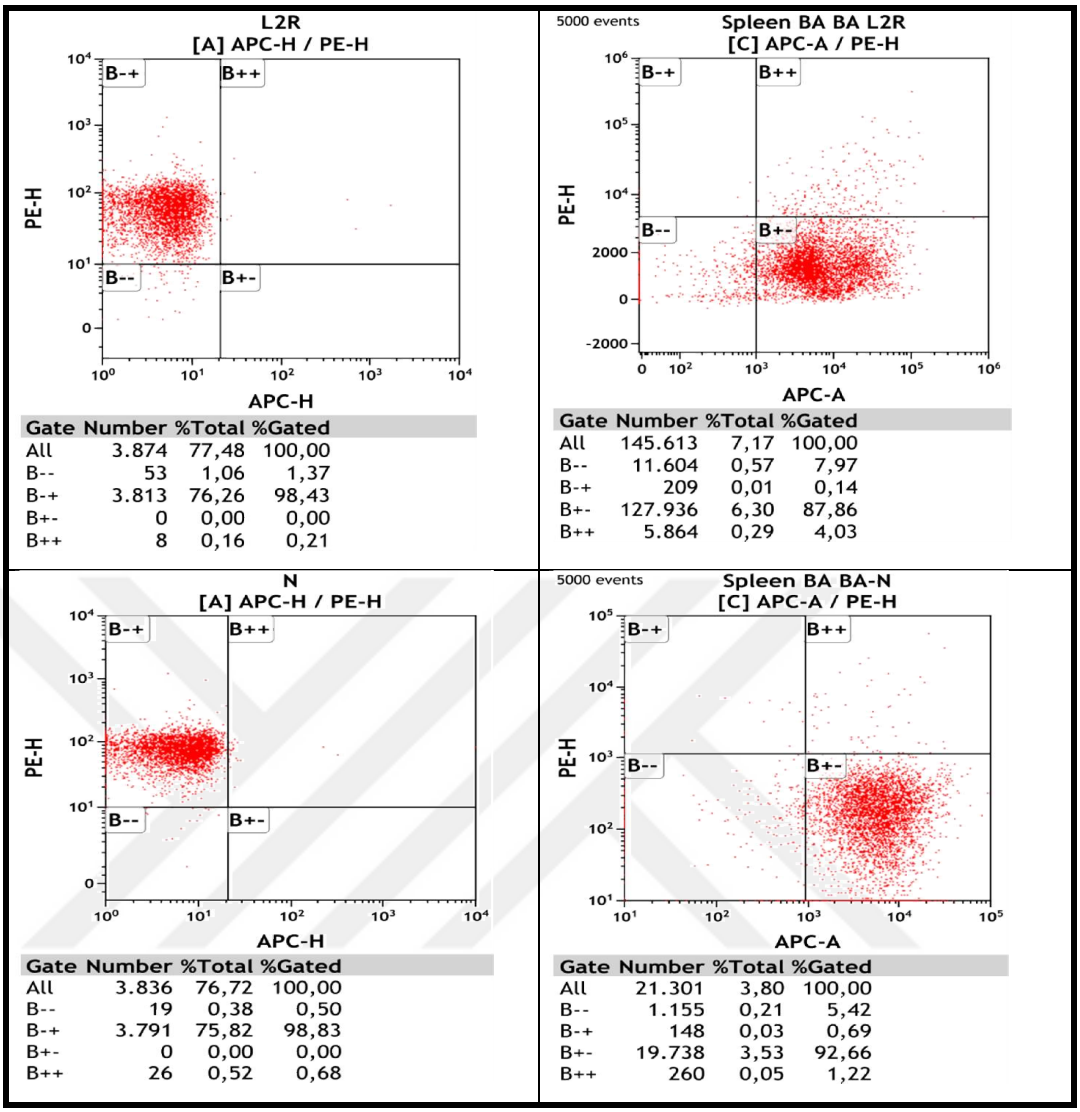
Peripheral blood (PB) was analyzed at 3, 4 and 6 month post-transplantation. The CD45.1 background host mice in which CD45.2 bone marrow cells have been transplanted show a chimeric-blood phenotype.

According to this results, 3th month after transplantation, chimeric-blood cells are 46, 82 percent LY/LY and 44, 00 percent BA/BA. At 4th month 37, 17 percent LY/LY and 60, 25 percent BA/BA of chimeric-blood cells were settled in recipient bone marrow. 40, 12 percent LY/LY and 56, 85 percent BA/BA of chimeric-blood cells were settled in recipient BM at 6th month.

4.3.4 After 500 rad, 1m ba/ba and ba/ly spleen transplantation

Figure 4.24: After 500 rad, transplantation with 1million BA/BA spleen cells





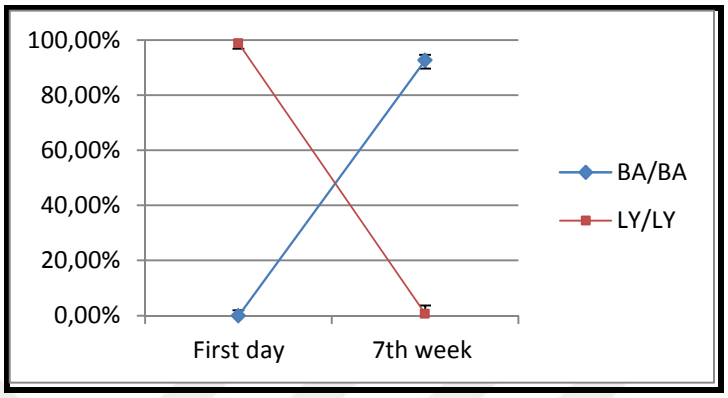
Reference: This figure was created by Deniz ALTUNSU.

Peripheral blood (PB) from transplanted-mice was analyzed at 7th week post-transplantation. According to the flow cytometer plots, donor ($CD45.1^+$) and competitor cells ($CD45.2^+$) is shifted for each lineage as shown.

These LY/LY (CD45.2) competitor mice were irradiated with 500 rad lethal dose. For a competitive transplant, BM cells from the CD45.1 were transplanted into the mouse originally. Bone marrow cells from donor mice retro-orbital injected into the irradiated recipient mice (CD45.2). PB was collected from tail of mice for the determination of the efficacy of bone marrow reconstitution. Peripheral blood (PB) was analyzed at 7th week post-transplantation. The CD45.2 background host mice in which CD45.1 bone marrow

cells have been transplanted show a chimeric-blood phenotype, however, at 7th week post-transplant.

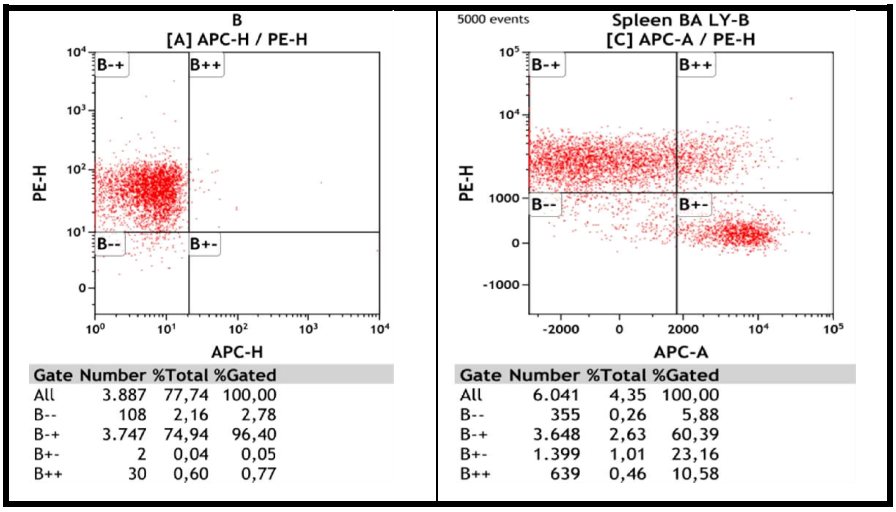
Figure 4.25: After ba/ba spleen transplantation, the percentage of ly/ly populations



Reference: This figure was created by Deniz ALTUNSU.

According to this figure, there is no any ba/ba population at first day. After ba/ba transplantation, the level of ba/ba populations were significantly increased and also ly/ly populations were dramatically decreased.

Figure 4.26: After 500 rad, transplantation with 1million BA/LY spleen cells

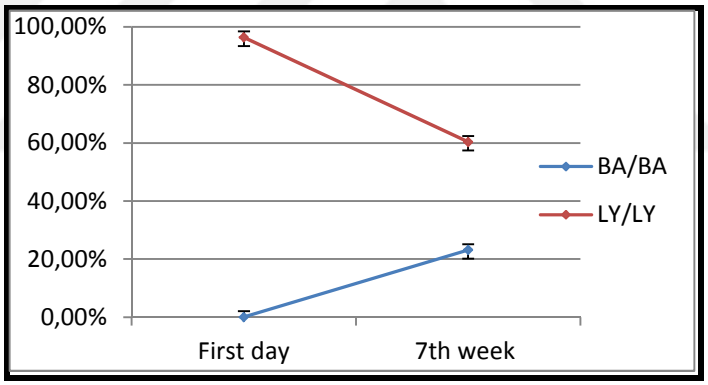


Reference: This figure was created by Deniz ALTUNSU.

PB was analyzed at 7th week post-transplantation. According to the flow cytometer plots, donor (CD45.2/CD45.1 mix cells) and competitor cells ($CD45.2^{+}$) is shifted for each lineage as shown.

These LY/LY (CD45.2) competitor mice were irradiated with 500 rad lethal dose. For a competitive transplant, 1:1 ratio of BM CD45.2:CD45.1 leukocytes, as they were transplanted into the mouse originally. Spleen cells from donor mice retro-orbital injected into the irradiated recipient mice (CD45.2). PB was collected from tail of mice for the determination of the efficacy of bone marrow reconstitution. Peripheral blood (PB) was analyzed at 7th week post-transplantation. The CD45.2 background host mice in which CD45.1 bone marrow cells have been transplanted show a chimeric-blood phenotype.

Figure 4.27: After ba/ba spleen transplantation, the percentage of ly/ly and ba/ba populations

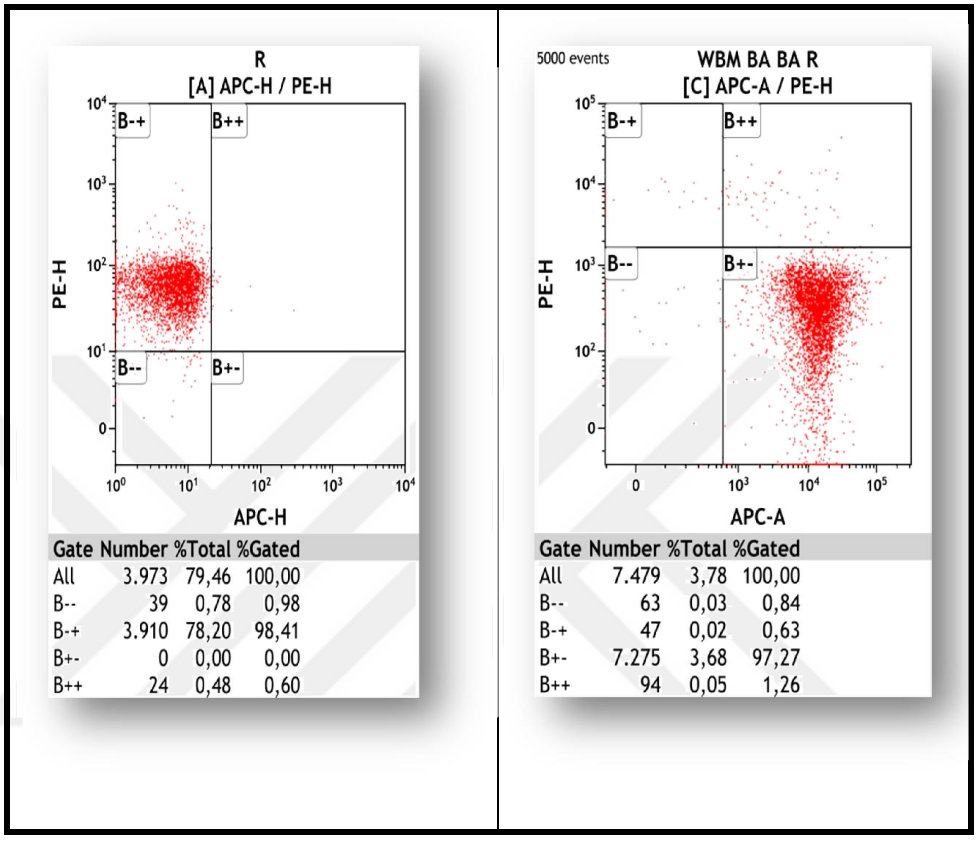


Reference: This figure was created by Deniz ALTUNSU.

According to this figure, there is no any ba/ba population at first day. After ba/ba transplantation, when the level of ba/ba populations were increased and also ly/ly populations were dramatically decreased. Post-transplantation in time, these populations are the same.

4.3.5 After 500 rad, 1m ba/ba wbm transplantation

Figure 4.28: After 500 rad, transplantation with 1million BA/BA wbm cells.



Reference: This figure was created by Deniz ALTUNSU.

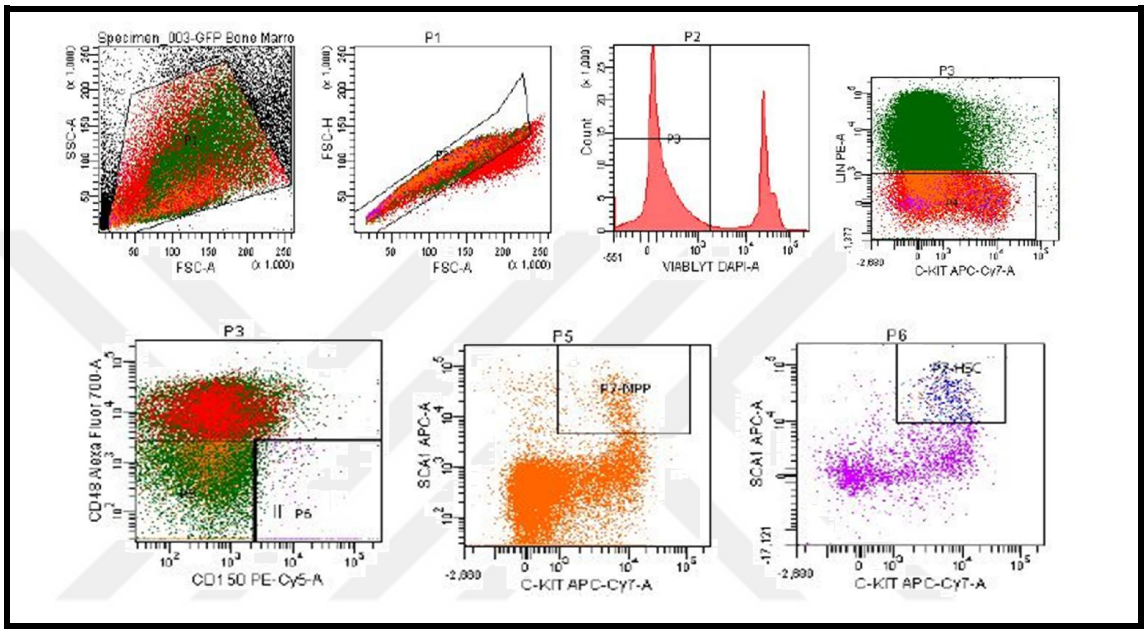
Peripheral blood (PB) from transplanted-mice was analyzed at 7th week post-transplantation. According to the flow cytometer plots, donor ($CD45.1^+$) and competitor cells ($CD45.2^+$) is shifted for each lineage as shown.

These LY/LY (CD45.2) competitor mice were irradiated with 500 rad lethal dose. For a competitive transplant, BM cells from the CD45.1 were transplanted into the mouse originally. Bone marrow cells from donor mice retro-orbital injected into the irradiated recipient mice (CD45.2). PB was collected from tail of mice for the determination of the efficacy of bone marrow reconstitution. Peripheral blood (PB) was analyzed at 7th week post-transplantation. The CD45.2 background host mice in which CD45.1 bone marrow

cells have been transplanted show a chimeric-blood phenotype, however, at 7th week post-transplant.

4.4 Investigation of hscs in bone marrow, spleen and spine bone

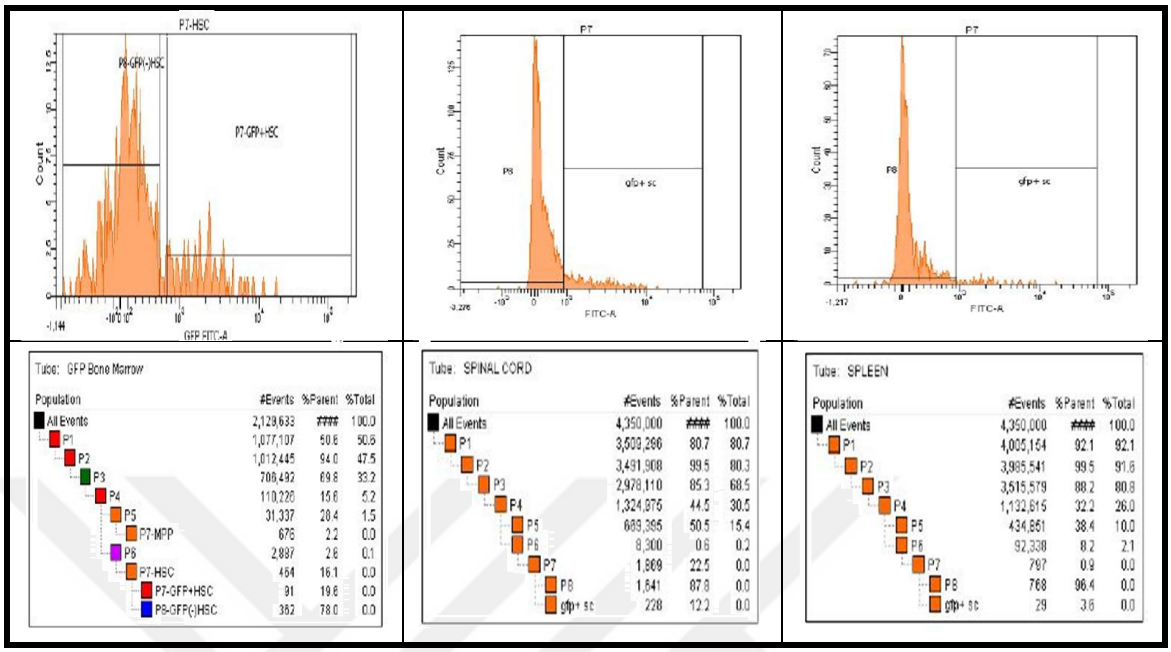
Figure 4.29: Sorting of a-catulin–GFP+ c-kit+ cells



Reference: This figure was prepared by Deniz ALTUNSU.

Firstly, all population was defined for sorting. And then single cells were selected. To identify live or dead cells, DAPI staining was used. By use of Lin PE-A and c-kit APC-Cy7-A antibodies, c-kit positive and lin negative cell populations were obtained. After, slam cell surface markers that CD150 and CD48 were used for detection of multipotent hematopoietic progenitors and hematopoietic stem cells.

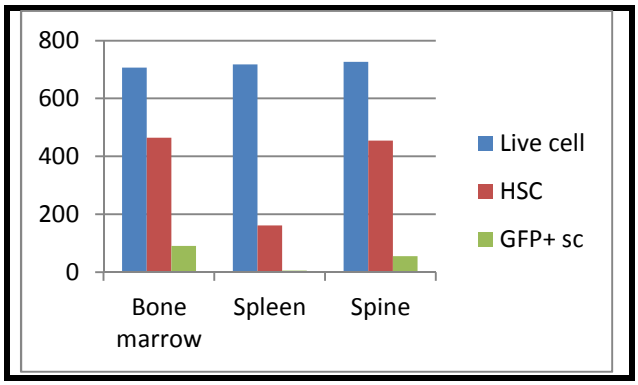
Figure 4.30: Analysis of GFP/+ hscs

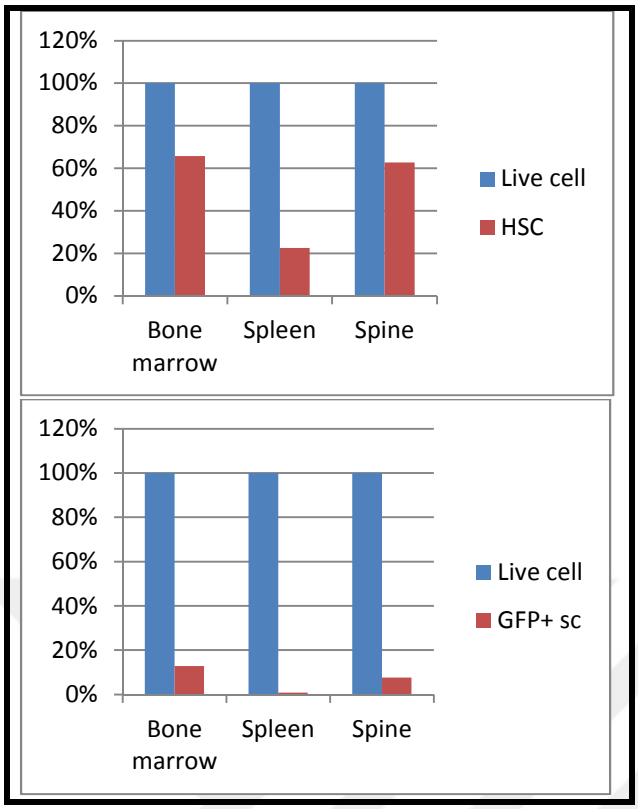


Reference: This figure was prepared by Deniz ALTUNSU

After selection of hspc and hsc, pure hsc which means that gfp/+ hscs were detected in bone marrow, spleen and spine bone.

Figure 4.31: Investigation of gfp/+ hscs in bm, spleen and spine bone





Reference: This figure was prepared by Deniz ALTUNSU.

According to flow cytometer results, 464 hscs were obtained in bone marrow, 162 hscs were obtained in spleen and also 455 hscs were obtained in spine bone. After this results, pure gfp/+ hscs were investigated. 12, 88 percent of gfp/+ hsc in bone, 0, 83 percent of gfp/+ hsc in spleen and 7, 57 percent of gfp/+ hscs in spine bone were obtained.

5. CONCLUSION

In conclusion, congenic Ly5.1/Ly5.2 murine transplantation model is reliable for such an experiment. This model is also satisfactory and useful for all transplantation studies. In addition, these mice support to study direct competition of two grafts. Conclusions obtained from both systems are well reproducible for both strain and sex combinations. In mice with total chimerism, the main WBC subpopulation of the graft is B-lymphocytes, as in normal mice. After radiation therapy, we transplanted WBM and spleen cells into myelo-suppressed mice. So we eliminated the effect of TBI and reconstitute hematopoietic system via 1 million spleen and WBM cells. By the using of congenic mice, we measured reconstitution of the blood system adult lineages post-irradiation in transplant recipient mice. In addition, we determined stemness in vivo by competitor assay and we distinguished donor from competitor cells upon transplantation. We claim that competitor mice are congenic and carry the differential B cell antigen originally designated CD45.1/Ly5.1.

REFERENCES

Periodical

Abkowitz, J. L., et al. (2002). "Evidence that the number of hematopoietic stem cells per animal is conserved in mammals." Blood **100**(7): 2665-2667.

Acar, M., et al. (2015). "Deep imaging of bone marrow shows non-dividing stem cells are mainly perisinusoidal." Nature **526**(7571): 126.

Adolfsson, J., et al. (2001). "Upregulation of Flt3 expression within the bone marrow Lin− Sca1+ c-kit+ stem cell compartment is accompanied by loss of self-renewal capacity." Immunity **15**(4): 659-669.

Aiuti, A., et al. (1999). "Expression of CXCR4, the receptor for stromal cell-derived factor-1 on fetal and adult human lymphohematopoietic progenitors." European journal of immunology **29**(6): 1823-1831.

Allman, D., et al. (2003). "Thymopoiesis independent of common lymphoid progenitors." Nature immunology **4**(2): 168.

Anasetti, C., et al. (2012). "Back to the future: mismatched unrelated donor, haploidentical related donor, or unrelated umbilical cord blood transplantation?" Biology of blood and marrow transplantation **18**(1): S161-S165.

Anasetti, C., et al. (2012). "Peripheral-blood stem cells versus bone marrow from unrelated donors." New England Journal of Medicine **367**(16): 1487-1496.

Andersson, B. S., et al. (2002). "Busulfan systemic exposure relative to regimen-related toxicity and acute graft-versus-host disease: defining a therapeutic window for iv BuCy2 in chronic myelogenous leukemia." Biology of blood and marrow transplantation **8**(9): 477-485.

Askenasy, N., et al. (2003). "Characterization of adhesion and viability of early seeding hematopoietic cells in the host bone marrow in vivo and in situ." Experimental hematology **31**(12): 1292-1300.

Atilla, E., et al. (2017). "A review of myeloablative vs reduced intensity/non-myeloablative regimens in allogeneic hematopoietic stem cell transplantations." Balkan medical journal **34**(1): 1.

Attar, A. (2014). Changes in the Cell Surface Markers During Normal Hematopoiesis: A Guide to Cell Isolation.

Berrios, V. M., et al. (2001). "The molecular basis for the cytokine-induced defect in homing and engraftment of hematopoietic stem cells." Experimental hematology **29**(11): 1326-1335.

Bhatia, M., et al. (1998). "A newly discovered class of human hematopoietic cells with SCID-repopulating activity." Nature medicine **4**(9): 1038.

Broxmeyer, H. E., et al. (2011). "Hematopoietic stem/progenitor cells, generation of induced pluripotent stem cells, and isolation of endothelial progenitors from 21-to 23.5-year cryopreserved cord blood." Blood **117**(18): 4773-4777.

Broxmeyer, H. E., et al. (2005). "Rapid mobilization of murine and human hematopoietic stem and progenitor cells with AMD3100, a CXCR4 antagonist." Journal of Experimental Medicine **201**(8): 1307-1318.

Calvi, L., et al. (2003). "Osteoblastic cells regulate the haematopoietic stem cell niche." Nature **425**(6960): 841.

Cavazzana-Calvo, M., et al. (2000). "Gene therapy of human severe combined immunodeficiency (SCID)-X1 disease." Science **288**(5466): 669-672.

Choi, K., et al. (1998). "A common precursor for hematopoietic and endothelial cells." Development **125**(4): 725-732.

Christensen, J. L. and I. L. Weissman (2001). "Flk-2 is a marker in hematopoietic stem

cell differentiation: a simple method to isolate long-term stem cells." Proceedings of the National Academy of Sciences **98**(25): 14541-14546.

Chute, J. P. (2006). "Stem cell homing." Current opinion in hematology **13**(6): 399-406.

Copelan, E. A. (2006). "Hematopoietic stem-cell transplantation." New England Journal of Medicine **354**(17): 1813-1826.

Craddock, C., et al. (1997). "The role of CS1 moiety of fibronectin in VLA4-mediated haemopoietic progenitor trafficking." British journal of haematology **97**(1): 15-21.

Domingues, M. J., et al. (2017). "New agents in HSC mobilization." International journal of hematology **105**(2): 141-152.

Gaspar, H. B., et al. (2011). "Long-term persistence of a polyclonal T cell repertoire after gene therapy for X-linked severe combined immunodeficiency." Science translational medicine **3**(97): 97ra79-97ra79.

Gerber, H.-P., et al. (2002). "VEGF regulates haematopoietic stem cell survival by an internal autocrine loop mechanism." Nature **417**(6892): 954.

Gumbiner, B. M. (1996). "Cell adhesion: the molecular basis of tissue architecture and morphogenesis." cell **84**(3): 345-357.

Guo, B., et al. (2016). Human Hematopoietic Stem Cell Homing and Engraftment Promoted By Glucocorticoid Induced Chromatin Remodeling, Am Soc Hematology.

Hacein-Bey-Abina, S., et al. (2003). "A serious adverse event after successful gene therapy for X-linked severe combined immunodeficiency." New England Journal of Medicine **348**(3): 255-256.

Hacein-Bey-Abina, S., et al. (2003). "LMO2-associated clonal T cell proliferation in two patients after gene therapy for SCID-X1." Science **302**(5644): 415-419.

Hanna, J., et al. (2007). "Treatment of sickle cell anemia mouse model with iPS cells generated from autologous skin." Science **318**(5858): 1920-1923.

Heath, J. L., et al. (2019). "The role of cell adhesion in hematopoiesis and leukemogenesis." Journal of cellular physiology.

Heissig, B., et al. (2005). "A role for niches in hematopoietic cell development." Hematology **10**(3): 247-253.

Joshi, R., et al. (2017). "Proteolytic fragments of fibronectin function as matrikines driving the chemotactic affinity of prostate cancer cells to human bone marrow mesenchymal stromal cells via the α5β1 integrin." Cell adhesion & migration **11**(4): 305-315.

Karantanou, C., et al. (2018). "Targeting the bone marrow microenvironment in acute leukemia." Leukemia & lymphoma **59**(11): 2535-2545.

Karlsson, S., et al. (2002). "Development of gene therapy for blood disorders by gene transfer into haematopoietic stem cells." Haemophilia **8**(3): 255-260.

Kohlscheen, S., et al. (2019). "Endothelial protein C receptor supports hematopoietic stem cell engraftment and expansion in Mpl-deficient mice." Blood **133**(13): 1465-1478.

Kolb, H.-J. (2008). "Graft-versus-leukemia effects of transplantation and donor lymphocytes." Blood **112**(12): 4371-4383.

Körbling, M. and P. Anderlini (2001). "Peripheral blood stem cell versus bone marrow allotransplantation: does the source of hematopoietic stem cells matter?" Blood **98**(10): 2900-2908.

Körbling, M. and E. J. Freireich (2011). "Twenty-five years of peripheral blood stem cell transplantation." Blood **117**(24): 6411-6416.

Kucia, M., et al. (2005). "Trafficking of normal stem cells and metastasis of cancer stem cells involve similar mechanisms: pivotal role of the SDF-1–CXCR4 axis." Stem cells **23**(7): 879-894.

Lapidot, T., et al. (2005). "How do stem cells find their way home?" Blood **106**(6): 1901-1910.

Lataillade, J. J., et al. (2004). "Stromal cell-derived factor-1 (SDF-1)\CXCR4 couple plays multiple roles on haematopoietic progenitors at the border between the old cytokine and

new chemokine worlds: survival, cell cycling and trafficking." European cytokine network **15**(3): 177-188.

Laterveer, L., et al. (1996). "Improved survival of lethally irradiated recipient mice transplanted with circulating progenitor cells mobilized by IL-8 after pretreatment with stem cell factor." Experimental hematology **24**(12): 1387-1393.

Lee, J., et al. (2017). "Lineage specification of human dendritic cell is marked by expression of the transcriptionl factor IRF8 in HSCs and MPPs." Nature immunology **18**(8): 877.

Lévesque, J.-P., et al. (2002). "Mobilization by either cyclophosphamide or granulocyte colony-stimulating factor transforms the bone marrow into a highly proteolytic environment." Experimental hematology **30**(5): 440-449.

Levesque, J.-P., et al. (2004). "Characterization of hematopoietic progenitor mobilization in protease-deficient mice." Blood **104**(1): 65-72.

Liles, W. C., et al. (2003). "Mobilization of hematopoietic progenitor cells in healthy volunteers by AMD3100, a CXCR4 antagonist." Blood **102**(8): 2728-2730.

Marjon, K. D., et al. (2016). "Tetraspanin CD82 regulates bone marrow homing of acute myeloid leukemia by modulating the molecular organization of N-cadherin." Oncogene **35**(31): 4132.

Matsuoka, S., et al. (2001). "CD34 expression on long-term repopulating hematopoietic stem cells changes during developmental stages." Blood **97**(2): 419-425.

Miller, P. H., et al. (2016). "Quantitation of Human Cells that Produce Neutrophils and Platelets in Vivo Obtained from Normal Donors Treated with Granulocyte Colony–Stimulating Factor and/or Plerixafor." Biology of blood and marrow transplantation **22**(11): 1945-1952.

Morrison, S. J. and I. L. Weissman (1994). "The long-term repopulating subset of hematopoietic stem cells is deterministic and isolatable by phenotype." Immunity **1**(8): 661-673.

Naderi-Meshkin, H., et al. (2015). "Strategies to improve homing of mesenchymal stem cells for greater efficacy in stem cell therapy." Cell biology international **39**(1): 23-34.

Orschell-Traycoff, C. M., et al. (2000). "Homing and engraftment potential of Sca-1+ lin− cells fractionated on the basis of adhesion molecule expression and position in cell cycle." Blood **96**(4): 1380-1387.

Ott, M. G., et al. (2006). "Correction of X-linked chronic granulomatous disease by gene therapy, augmented by insertional activation of MDS1-EVI1, PRDM16 or SETBP1." Nature medicine **12**(4): 401.

Papayannopoulou, T. and B. Nakamoto (1993). "Peripheralization of hemopoietic progenitors in primates treated with anti-VLA4 integrin." Proceedings of the National Academy of Sciences **90**(20): 9374-9378.

Petit, I., et al. (2002). "G-CSF induces stem cell mobilization by decreasing bone marrow SDF-1 and up-regulating CXCR4." Nature immunology **3**(7): 687.

Pinho, S. and P. S. Frenette (2019). "Haematopoietic stem cell activity and interactions with the niche." Nature Reviews Molecular Cell Biology: 1.

Qin, Y., et al. (2016). "Bone marrow stromal/stem cell-derived extracellular vesicles regulate osteoblast activity and differentiation in vitro and promote bone regeneration in vivo." Scientific reports **6**: 21961.

Rekers, P. E., et al. (1950). "Effect of transplantation of bone marrow into irradiated animals." Archives of Surgery **60**(4): 635-667.

Rivière, I., et al. (2012). "Hematopoietic stem cell engineering at a crossroads." Blood **119**(5): 1107-1116.

Robinton, D. A. and G. Q. Daley (2012). "The promise of induced pluripotent stem cells in research and therapy." Nature **481**(7381): 295.

Sackstein, R. (2016). Methods to improve cell therapy, Google Patents.

Salam, S. G. R. A., et al. (2017). "Efficacy of combined administration of chemoimmunotherapy with bone marrow cells or granulocyte-colony stimulating factor-

mobilized stem cells on expansion of myeloid and stem cells." Clinical Cancer Investigation Journal **6**(1): 73.

Sato, N., et al. (1994). "A time course study for optimal harvest of peripheral blood progenitor cells by granulocyte colony-stimulating factor in healthy volunteers." Experimental hematology **22**(10): 973-978.

Satwani, P., et al. (2008). "Reduced intensity and non-myeloablative allogeneic stem cell transplantation in children and adolescents with malignant and non-malignant diseases." Pediatric blood & cancer **50**(1): 1-8.

Schofield, R. (1978). "The relationship between the spleen colony-forming cell and the haemopoietic stem cell." Blood cells **4**(1-2): 7-25.

Servais, S., et al. (2011). "Allogeneic hematopoietic stem cell transplantation (HSCT) after reduced intensity conditioning." Transfusion and Apheresis Science **44**(2): 205-210.

Spangrude, G. J., et al. (1988). "Purification and characterization of mouse hematopoietic stem cells." Science **241**(4861): 58-62.

Stein, J., et al. (2005). "Critical early events in hematopoietic cell seeding and engraftment." Folia histochemica et cytobiologica **43**(4): 191-195.

Sundaramoorthy, P., et al. (2017). "Thioredoxin mitigates radiation-induced hematopoietic stem cell injury in mice." Stem cell research & therapy **8**(1): 263.

Szelinski, F., et al. (2018). FRI0275 Altered protein tyrosine phosphatases activity and disease specific expression pattern of cd45 and cd148 on various lymphocyte subsets in autoimmune diseases, BMJ Publishing Group Ltd.

Szodoray, P., et al. (2016). "T-helper signals restore B-cell receptor signaling in autoreactive anergic B cells by upregulating CD45 phosphatase activity." Journal of Allergy and Clinical Immunology **138**(3): 839-851. e838.

Takahashi, K. and S. Yamanaka (2006). "Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors." cell **126**(4): 663-676.

Takakura, N., et al. (1998). "Critical role of the TIE2 endothelial cell receptor in the development of definitive hematopoiesis." Immunity **9**(5): 677-686.

Thomas, E. D. (1999). "A history of haemopoietic cell transplantation." British journal of haematology **105**(2): 330-339.

Thomas, E. D., et al. (1957). "Intravenous infusion of bone marrow in patients receiving radiation and chemotherapy." New England Journal of Medicine **257**(11): 491-496.

Tiercy, J.-M. (2016). "How to select the best available related or unrelated donor of hematopoietic stem cells?" Haematologica **101**(6): 680-687.

Till, J. E. and E. A. McCulloch (1961). "A direct measurement of the radiation sensitivity of normal mouse bone marrow cells." Radiation research **14**(2): 213-222.

Verfaillie, C. (1998). "Adhesion receptors as regulators of the hematopoietic process." Blood **92**(8): 2609-2612.

Visnjic, D., et al. (2004). "Hematopoiesis is severely altered in mice with an induced osteoblast deficiency." Blood **103**(9): 3258-3264.

Wang, H., et al. (2018). "Fast recovery of platelet production in NOD/SCID mice after transplantation with ex vivo expansion of megakaryocyte from cord blood CD34+ cells." Journal of cancer research and therapeutics **14**(1): 233.

Warburton, D., et al. (2008). "Stem/progenitor cells in lung development, injury repair, and regeneration." Proceedings of the American Thoracic Society **5**(6): 703-706.

Wernig, M., et al. (2008). "Neurons derived from reprogrammed fibroblasts functionally integrate into the fetal brain and improve symptoms of rats with Parkinson's disease." Proceedings of the National Academy of Sciences **105**(15): 5856-5861.

Willasch, A. M., et al. (2017). Myeloablative conditioning for first allogeneic hematopoietic stem cell transplantation in children with all: total body irradiation or chemotherapy?-a multicenter EBMT-PDWP Study, Am Soc Hematology.

Winkler, I. G. and J.-P. Levesque (2006). "Mechanisms of hematopoietic stem cell

mobilization: when innate immunity assails the cells that make blood and bone." Experimental hematology **34**(8): 996-1009.

Wright, D. E., et al. (2001). "Physiological migration of hematopoietic stem and progenitor cells." Science **294**(5548): 1933-1936.

Yang, L., et al. (2018). "Roles of integrin αvβ3 in SDF-1/CXCR4-induced choroidal neovascularization." Recent Advances in Ophthalmology **38**(5): 401-406.

Yilmaz, Ö. H., et al. (2006). "SLAM family markers are conserved among hematopoietic stem cells from old and reconstituted mice and markedly increase their purity." Blood **107**(3): 924-930.

Zahler, S., et al. (2016). "A phase I study of reduced-intensity conditioning and allogeneic stem cell transplantation followed by dose escalation of targeted consolidation immunotherapy with gemtuzumab ozogamicin in children and adolescents with CD33+ acute myeloid leukemia." Biology of blood and marrow transplantation **22**(4): 698-704.

Zhang, J., et al. (2014). "Small molecule Me6TREN mobilizes hematopoietic stem/progenitor cells by activating MMP-9 expression and disrupting SDF-1/CXCR4 axis." Blood **123**(3): 428-441.

Ziegler, B., et al. (1999). "KDR receptor: a key marker defining hematopoietic stem cells." Science **285**(5433): 1553-1558.