Enhancement of hematopoiesis and thrombopoiesis by pharmacological compounds valproic acid and all- trans retinoic acid.

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Abstract

The two processes , Megakaryopoiesis and Thrombopoiesis are important from a clinical perspective. Abnormalities in these processes lead to conditions such as thrombocytopenia, for which no specific and accurate treatments are available.In umbilical cord blood transplantation the megakaryocyte progenitor population is less and its engraftment is delayed, thus there is a need of *ex-vivo* expansion of these progenitors for better platelet recovery .Methods of *in vitro* generation of megakaryocytes (MKs) and platelets serve as a good tool to understand the process of platelet biogenesis and also to overcome hurdles in platelet transfusion. Pharmacological compounds like Thrombopoietin (TPO) mimetics (eltrombopag), HDAC inhibitors (Valproic acid, VPA) and vitamin A derivative (all-trans retinoic acid, ATRA) are being used to enhance the production of platelets. This kind of study will contribute in understanding the basic cellular and molecular events taking place during megakaryopoiesis and thrombopoiesis and will also give us insight on clinical treatment of prolonged thrombocytopenia during stem cell transplant and other platelet disorders. In this review, we will mainly discuss about the importance of different sources, strategies and use of pharmacological compounds like VPA and ATRA in MK and platelet generation.

Keywords: Megakaryopoiesis, thrombopoiesis, Platelet, thrombocytopenia, all-trans retinoic acid and Valproic acid.

Abbreviations: Haematopoietic stem cells (HSC), Thrombopoietin (TPO), myeloproliferative leukemia protein (c-Mpl),megakaryocytes (MKs), Histone deacetylase inhibitor (HDAC), Valproic acid (VPA), all-trans retinoic acid (ATRA), Induced pluripotent stem cells (iPSC), Embryonic stem cells (ESC) , myelodysplastic syndrome (MDS), acute myeloid leukemia (AML),acute promyelocytic leukemia (APL), 5-Azacytidine (5-AZA), stem cell leukemia (SCL), T cell acute lymphocytic leukemia -1(TAL-1) and poly unsaturated fatty acid (PUFA).

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Journey from HSCs to megakaryocyte and Platelets-

Haematopoietic stem cells are the most extensively studied adult stem cells which have the potential to differentiate into cells of the blood lineages [1]. Where symmetric cell division is necessary for stem cell expansion, the asymmetric division gives rise to common lymphoid and common myeloid progenitor [2]. The latter further terminally differentiates into erythroid and megakaryocyte lineage on getting cues from growth factors like erythropoietin (EPO) and thrombopoietin (TPO) respectively on activation of specific transcription factors [3-4]. Megakaryocyte precursors develop and migrate from

osteoblastic niche to vascular niche during the differentiation into Megakaryocytes (MKs) and platelets [5]. MKs are giant multinucleated cells 100-150 micron in size which undergo, endoduplication , cytoplasmic maturation and form pseudopodia like extensions known as pro-platelet which releases platelets from their ends into the blood stream and the remaining MK cell body undergoes apoptosis (Figure 1 and 2)[6-14].

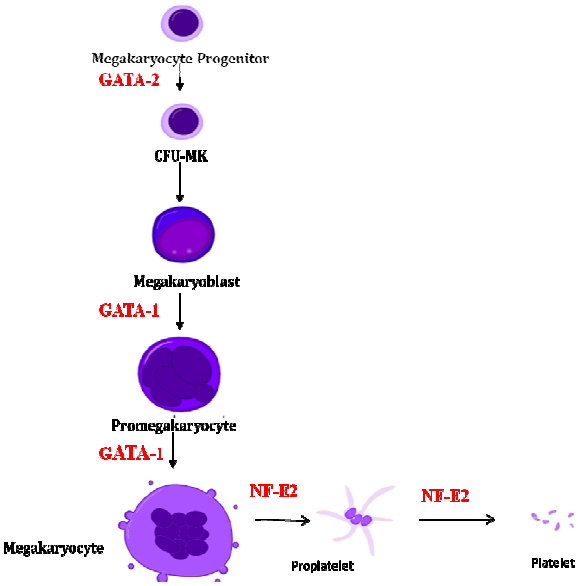

Figure 1: Schematic representation of differentiation of Megakaryocyte progenitor to megakaryocyte and platelet regulated by specific transcription factors i.e. GATA-1, GATA-2 and NF-E2.

The process of platelet biogenesis -

Megakaryocytes transit from immature cells to mature cells to release platelets [15]. This process involves steps of complex events taking place in a coordinated manner controlled by various cellular and molecular factors as depicted in fig. 2. The cells first undergo cytoplasmic maturation and expansion followed by nuclear endomitosis and organelle synthesis. Centrosomes are arranged at the centre emanating microtubule array [16]. Then, centrosomes disassemble and microtubules translocate to the cell cortex [17]. After this step thick pseudopod like structures are formed termed as proplatelets [18]. Organelles are translocated via sliding of microtubules. The proplatelet ends undergo bending and branching to expand and amplify the process of platelet formation and release [19-21]. The cell undergoes complete cytoplasmic remodelling and is converted into mass of proplatelets that release platelets into the blood stream whereas the nucleus undergoes apoptosis**.**

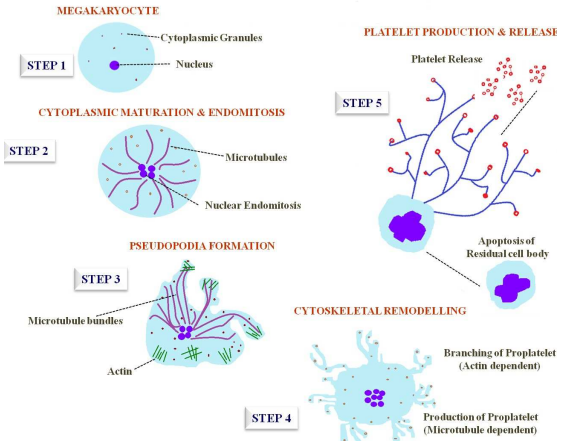

Figure 2: Stepwise representation of Platelet biogenesis.

The process of Megakaryopoiesis is a highly regulated process at both cellular and molecular levels however only a few transcription factors and signalling molecules are reported so far. Multiple transcription factors including Runx1, Gata1, Fli1 and c-Myb form complex networks that regulate the differentiation of megakaryocytes both positively and negatively. Two models have been proposed to explain platelet formation from mature megakaryocytes. In the fragmentation model, megakaryocytes travel from the bone marrow to the lung where they are broken up into platelets in the microvasculature [22]. Alternatively, in the proplatelet model, megakaryocytes in the bone marrow develop multiple branching processes that extend into the marrow sinusoids to release platelets into the circulation [23]. Multiple growth factors support

Megakaryopoiesis, the most important of which is thrombopoietin (TPO). The TPO receptor c-Mpl was identified based on its homology to the oncogne v-Mpl.TPO signalling depends on the activation of Jak2. Active Jak2 then phosphorylates itself on multiple residues and phosphorylates c-Mpl on at least Tyr625 and Tyr630. Following the activation of Jak2, multiple signaling molecules are activated and mediate the cellular response to TPO. These include members of the signal transducer and activator of transcription (STAT), mitogen-activated protein kinase (MAPK) and phosphoinositol-3 kinase (PI3K) pathways [24-26]. AK2 also activates the small GTPase Ras and the MAPK cascade, helping in the activation of extracellular signal-related kinase (ERK)1/2. Notch is a positive regulator of Megakaryopoiesis and plays a more complex role in cell-fate decisions among myeloid progenitors [27]. Megakaryocyte generates platelet through different stages of maturation .However the terminal steps of platelet release, that involve specific cellular, molecular and cytoskeletal changes are still unexplored and needs to be studied in detail.

Clinical importance of Platelets-

Platelets are small (2-3 micron) anucleate particles that have highly organised cytoskeleton and have abundant secretory granules. 10^{11} platelets are released daily into the blood stream of an adult human on a steady state basis and the numbers can increase or decrease under various disease / clinical conditions [28-29]. They play a very important role in maintaining haemostasis by activation and aggregation at the site of vessel injury and formation of blood clot [30-31].

The basic biological function of platelets is maintaining haemostasis [32]. They prevent haemorrhage after tissue injury by activation and formation of clot at that place. During injury the endothelial cells are damaged, blood leaks out of blood vessel and the platelets are activated by components of the extracellular matrix such as von Willebrand factor (vWF) and collagen. The platelet receptors like glycoprotein VI (GPVI) and glycoprotein Ibα (GPIbα) bind to collagen and von Willebrand factor (VWF), leading to activation of platelet integrin αIIbβ3 ,which helps in formation of strong adhesion and platelet aggregation[33-34]. The serine protease thrombin activates the platelets, converting soluble fibrinogen into insoluble fibrin. This maximizes local thrombus formation and the release of platelet granules that contain active pro-teases, growth factors, matrix proteins and chemokines [35-36]. This overall process is highly coordinated and maintains haemostasis in the body.

The normal platelet count ranges from 1, 50,000 to 400,000 per µl and any abnormality leads to either increase (Thrombocythemia) or decrease (Thrombocytopenia) in platelet count creating serious problems to human health. Various clinical conditions including immune thrombocytopenic purpura(ITP) [37], myelodysplastic syndromes (MDS)[38], chemotherapy-induced thrombocytopenia, continuous bleeding due to defect in blood clotting, aplastic anaemia, human immunodeficiency virus (HIV) infection are accompanied by thrombocytopenia in which the platelet count goes below $150x10^3$. Treatment for these conditions requires platelet transfusion which has certain drawbacks like the low shelf life and the reduced efficacy of platelets; moreover, it is expensive, time consuming, has high risk of carrying infections[39-47].Thus, there is a need to search new pharmacological compounds that can enhance the platelet production to overcome these hurdles of platelet transfusion.

Regulation of Platelet biogenesis by Thrombopoietin and other chemical modulators -

Thrombopoietin (TPO) is a major regulator of platelet biogenesis and HSC maintenance. It binds to myeloproliferative leukemia protein (c-Mpl) receptor present on MKs and HSCs. Studies performed in mice show that any defect in TPO, or its receptor, leads to a decrease in MK and platelet number, which becomes lethal in some cases. Any mutation in c-Mpl receptor leads to a serious condition known as congenital amegakaryocytic thrombocytopenia in infants. It clearly shows the importance of TPO in the process of megakaryopoiesis [48-50]. Discovery of TPO has been of immense importance as it leads to the *ex-vivo* generation of MK and platelets for clinical applications and research purposes [51-53]. TPO mimetics like recombinant-TPO and pegylated recombinant human megakaryocyte growth and development factor (PEG-rhMGDF) have been an alternative source used for increasing the platelet production. But these mimetics in comparison to TPO takes longer time for generation of MK and platelets from HSCs. Recombinant human TPO and PEG-rhMGDF were formulated as first generation TPO mimetics but had certain drawbacks. PEG-rhMGDF increased the platelet number in Non-myeloblative patients but there was no effect in myeloablative patients. During clinical trials it was observed that in some healthy volunteers recombinant TPO developed auto-antibodies targeting endogenous TPO, therefore the use of recombinant TPO was discontinued. Screening for high affinity peptides led to development of second generation TPO agonist such as romiplostim that binds to TPO receptor and the other is a non-peptide agonist eltrombopag that binds to transmembrane portion of TPO. Both of them are approved by FDA for treatment of immune thrombocytopenia (ITP) due to their improved pharmacological behaviour [54-55].

***In vitro* generation of megakaryocyte and Platelets -**

MKs are rare cells present in less than 1% population of all the hematopoietic cells in the bone marrow. MKs and platelets have been derived from various sources like CD34+ cells obtained from Umbilical cord blood, mobilized peripheral blood and bone marrow [56-57]. Umbilical cord blood proves to be an effective alternative source of HSCs during transplantation as it is a clinical waste, has a non-invasive mode of collection and shows less chances of graft versus host disease. Thrombocytopenia is a major problem occurring during chemotherapy, radiotherapy and which is overcome by transplanting expanded MK progenitors with unmanipulated HSCs after an umbilical cord blood cell transplantation, to reduce delayed platelet recovery [58]. Platelet transfusion is another strategy which also has certain drawbacks as mentioned above. Therefore in order to study the process of thrombopoiesis it is essential to produce these cells *in vitro* in large quantities.

Cellular and molecular studies related to megakaryopoiesis have been difficult due to two factors i.e. low number of megakaryocytic progenitors obtained and asynchronous cultures. Due to recent technical advancement, protocols have been designed to get pure MK precursors directly or by *ex-vivo* expansion from HSCs. Nowadays cell line models derived from leukaemia patients are used to study megakaryopoiesis [59]. MEG-01 is one such well established Megakaryoblast cell line obtained from chronic myelogenous leukemia patient. This cell line is a very useful model system for studying megakaryopoiesis and biosynthesis of MK lineage proteins [60-61]. Many research groups have used Phorbol diesters (Phorbol Myristate acetate, PMA) for MEG-01 differentiation to shed some light on underlying molecular mechanism of platelet production [62-63]. Other researchers have used liquid crystal related compounds that have similarity to cell membrane and have biological activity to induce platelet production from peripheral blood CD34+ cells

[64]. Additives like nicotinamide (vitamin B3)[65], heparin[66],proteoglycans [67], prostaglandins like 15-deoxy-delta-PGJ2 [68], covalently immobilized glycosaminoglycans [69], parthenolide derived from Feverfew plant [70] when added in the differentiation media in addition to growth factors enhance MK and platelet production from primary cells or cell lines. Synthetic oleane triterpenoids-CDDO methyl ester having various pharmacological properties also enhances platelet production from HSC precursors [71]. Their derivatives are already under clinical trials for treating cancer. Many research groups are trying to screen various compounds which are biologically active and increase the platelet production *in vitro* for use in clinical trials to treat thrombocytopenic conditions.

Platelet transfusion can lead to generation of auto-antibodies against HLA and HPA of allogeneic transfused platelets in multiply transfused patients [72]. To overcome these hurdles nowadays platelets are being generated from iPSCs and ESCs as platelets are devoid of nuclei, they can be irradiated before transfusion to avoid any iPSC cell contamination leading to tumor formation [73-75]. A serum and feeder layer free differentiation medium has also been designed to obtain MKs and platelets from embryonic stem cells through spin embryoid body formation [76]. A new concept of iMKs (induced megakaryocyte) was developed by Masturba and colleagues in which mouse and human fibroblasts were directly transdifferentiated to MKs by transfection with three factors p45NF-E2 (transcription factor that regulates MK terminal differentiation) and Maf G, and Maf K (binding partners of p45NFE2). iMKs have an advantage of direct differentiation, taking only 2 weeks for generation of platelets as compared to 2 months by iPSC or ESC. But iMKs have a disadvantage of producing low number of platelets per MK as compared to iPSCs derived platelets [77].Human endometrial stromal stem cells have also shown their capacity to form MK and platelet, as an autologous source in regenerative therapy[78].As MKs are produced in bone marrow, the niche also supports their proliferation and differentiation. This has been shown in one of the studies where it was seen that the generation of MKs and platelets from HSCs is favoured when the cells are expanded on mesenchymal stem cells [79]. Recently, role of micro-RNAs has been revealed in increasing the efficiency of platelet production ex-vivo. It was found that miR-125b and miR-660 control polyploidization and the miR-23 a/27a/24-2 cluster together regulates platelet formation negatively [80].

Historical background for use of two pharmacological agents i.e. all trans retinoic acid (ATRA) and valproic acid (VPA) in clinics -

Various sources including primary cells and cell line models [81] described above are used for studying the cellular and molecular events underlying platelet biogenesis. Differentiation of HSCs to MKs and platelets have been studied by using combinations of growth factors like TPO, SCF, IL6 , IL11,specific transcription factors and therapeutic compounds. Here we are mainly focussing on the use of ATRA and VPA in clinical trials for treatment of various disorders that give a strong evidence for their active pharmacological behaviour.

ATRA is derived from vitamin A and is known to play important role during development as it helps in maturation and differentiation of hematopoietic precursor cells. Its deficiency leads to anaemia and treatment requires retinoic acid supplementation. ATRA is known for its pharmacological use in acute promyelocytic leukemia (APL), dose ranging from 10^{-8} M to 10^{-6} M [82]. The treatment is more effective when combined with chemotherapy drugs. ATRA binds to retinoic acid receptor whereas

its natural isoform 9-cis- retinoic acid binds to both retinoid acid receptor (RAR) as well as rexinoid receptor (RXR) and facilitates gene transcription via RAR elements. ATRA synergises its effect by activating other signalling pathways like ERK and P38 MAP kinase pathway.

VPA is a short chain fatty acid used as an anticonvulsant for the treatment of epilepsy and bipolar disorders since more than last 20 years [83-85]. It is a known class I/II HDAC (histone deacetylase) inhibitor. Gene expression is controlled by Histone acetylases and deacetylases [86-88]. In normal cells their activity is balanced, but in malignant cells HDAC's show abnormal activity and deacetylate tumour suppressor genes making them inactive, leading to progression of cancer.VPA causes growth arrest and differentiation of cancer cells *in vitro*[89]. But whether this activity is mediated by inhibition of HDAC or some other target needs to be studied in detail. VPA can induce differentiation of cell lines e.g. neuroblastoma, and teratocarcinoma [90] and also of leukemic blasts from acute myeloid leukemia patients. It has been shown that VPA acetylates tumour suppressor genes and facilitates proliferation, differentiation and apoptosis in malignant cells as shown in different cell line models *in vitro*. Earlier phenyl butyrate was used as HDAC inhibitor for the treatment of myelodysplastic syndrome and acute myelogenous leukemia but the response gained was not successful. VPA alone and in combination with ATRA was subsequently used in clinical study carried out by Kuendgen et al. for the treatment of MDS (myelodysplastic syndrome) and AML (acute myeloid leukaemia) patients. The volunteers responded well, except a few that showed symptoms of vertigo and thrombocytopenia which was reduced after the treatment was stopped. VPA alone is effective and addition of ATRA further enhances its effect when given at a later stage [91]. VPA was used at the same concentration that was used for treating Seizures (neurological disorder) i.e. 50 -100µg/ml and concentration above this is considered cytotoxic. These properties of low toxicity, stable serum concentration and local mode of application make VPA a potent therapeutic compound.

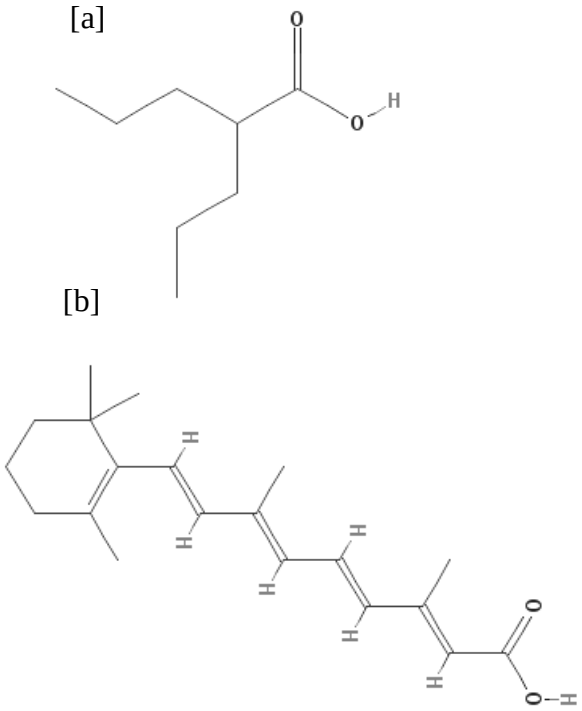

Figure 3: Chemical structure of [a] Valproic acid (VPA), chemical name- 2-Propylpentanoic acid, chemical formula $C_8H_{16}O_2$, *[b] all-trans retinoic acid (ATRA), chemical name- Retinoic acid, chemical formula* $C_{20}H_{28}O_2$. *(Adapted from NCBI, Pub chem. compound database)*

In early nineties, various research groups have studied the effect of ATRA on hematopoietic system. It showed differential behaviour towards precursor cells depending upon their specific lineage, it was either stimulatory or inhibitory .Various experiments were carried out to decipher the mode of action of ATRA to see whether it was an individual effect or a combined effect mediated via some internal factors [92-93]. To increase the efficacy of VPA and ATRA in terms of rate of action, Soriano et al. has

exploited the use of DNA hypomethylating agent 5-AZA (5-azacytidine) in patients suffering from acute myeloid leukemia ,high risk myelodysplastic syndrome and ATRA-resistant APL (acute promyelocytic leukemia). DNA methylation is a process in which a methyl group is added to a cytosine residue followed by a guanine residue. They are in repressed conditions in human genome except in the CpG islands present near gene promoters. In cancer, these CpG islands undergo aberrant methylation leading to gene suppression, which is overcome by DNA methyl transferase inhibitors that hypomethylate them and restore gene function [94-95]. Therefore the combinatorial therapy of HDAC inhibitors and hypomethylating agent was exploited in clinical trials for AML and MDS patients. In this study VPA and 5-AZA was given for 7 days daily, VPA at three different doses 50, 62.5, and 75 mg/kg orally and 5-AZA at 75 mg/m^2 subcutaneous respectively whereas ATRA was given from 3^{rd} day to 7^{th} day (i.e. only for 5 days) at a concentration of 45 mg/m^2.Out of these doses 50 mg/kg of VPA worked best with 5 AZA and ATRA. The cytoxicity still remains an issue with VPA as it is the least effective HDAC inhibitor and if the dose is increased it creates toxicity. There is a need to study the combination of 5-AZA and ATRA with a more effective and less toxic HDAC inhibitor in comparison to VPA. Administration of valproic acid and 5-AZA sensitizes ATRA resistant APL patients to respond to ATRA and gives better results [96]. Low frequency of side effects and total reversal of haematological remission was seen for small group of AML patients when treated with continuous valproic acid and intermittent ATRA plus low-dose of cytarabine. This suggests that a combinatorial therapy works better than VPA alone [97].VPA is a very well known anti-epileptic drug used for a very long time. It was used for studying the growth and differentiation of human acute promyelocytic leukemia (APL) cell line NB4 at the same dose used for epileptic treatment. It was also tried in combination with ATRA. After 48 hrs of treatment, both ATRA and VPA alone induced differentiation in NB4 cells, and their combination further enhanced the differentiation process. There was an upregulation of transcription factors i.e. CCAAT/enhancer-binding proteins (CEBP α, β, ε) and PU.1, that control normal myelopoiesis but are in a repressed condition in APL .Thus VPA and ATRA treatment restores normal transcriptional machinery for myelopoiesis which is the possible molecular mechanism behind the combinatorial beneficial effect [98]. Further studies can lead to development of new compounds which provide better treatment regimes in addition to these factors.

Effect of valproic acid and ATRA on Hematopoiesis and megakaryopoiesis -

VPA and ATRA are therapeutic compounds used in clinical trials for the treatment of various haematological disorders, mainly because they induce differentiation. This property can also be used in research to bring about differentiation of HSCs to the desired cell type. In this part of the review we are mainly focussing on the use of valproic acid and ATRA in HSC maintenance, MK and platelet development studies.

The role of histone deacetylase inhibitors in normal hematopoiesis was explored by studying the effect of valproic acid on proliferation and differentiation of hematopoietic stem cells. VPA and its analogs differentiate hematopoietic cell line by inhibiting histone deacetylases and promote acetylation of histones of specific genes linked with differentiation. In one of the study it was shown that VPA acts by acetylation of histones linked with p21 promoter during HSCs differentiation [99]. Bug et al. has demonstrated that human CD34+ cells obtained from bone marrow and umbilical cord blood showed an increase in number

upon valproic acid treatment in a dose dependent manner (30-150µg/ml).It did not show any differentiation potential but increased proliferation as depicted by the cell cycle profile. There was an increase in the number of cells in S phase in VPA treated cells as compared to control; accompanied by reduction in expression of CDK inhibitor p21cip-1/waf-1 .The mode of valproic acid signalling for self renewal of HSCs was mediated by GSK-3β. VPA is known to phosphorylate GSK3β at Ser 9 and stabilize β-catenin which up-regulates HOXB4, a known target of β-catenin and primary regulator of self renewal [100]. Thus, VPA induces HSCs in cycling phase from quiescent stage which renders them more sensitive towards chemotherapeutic and other pharmacological drugs for treatment in haematological malignancies. When HSCs were grown in semi-solid cultures with specific growth factors and with different concentrations of ATRA, it was found that ATRA promoted differentiation of HSCs to granulocytic, erythroid or megakaryocytic progenitors. It promoted MK lineage at low concentration and inhibited CD34+ proliferation at higher concentration [101]. Therefore, ATRA can be used along with TPO or other growth factors to study megakaryopoiesis.

In another study carried out by De felice et al. they used two types of growth factor cocktails, one was FLT3L and TPO and the other was FLT3L, SCF, TPO and IL-3, for *ex vivo* expansion of $CD34^+$ cells. When this study was combined with VPA it enhanced the expansion of $CD34^+$ cells. It was mediated by H4 histone acetylation at AC133 and HOXB4 regulatory sites [102]. This kind of study highlights new approaches for stem cell expansion and proliferation by modulating epigenetic regulation by different therapeutic agents. HSCs obtained from umbilical cord blood are utilised in stem cell transplantation, but their number is limited causing delayed engraftment. Therefore, new strategies are required to increase their engraftment potential. Homing of hematopoietic stem cells to bone marrow niche is mediated by SDF1-α, which is secreted by bone marrow stromal cells and which acts on CXCR4 receptor present on $CD34^+$ cells. It was observed that VPA enhances CXCR4 expression on HSCs and increases their engraftment potential in NOD-SCID mice [103]. VPA is already known for proliferation and self renewal potential of HSCs. This property was used in conjunction with the selective amplification technology developed by Viacell Inc. When frozen stem cells were expanded for 7 days in fresh medium in the presence of VPA it enhanced the $CD45^+34^+$ populations by 2 fold as compared to control and resulted in a 2.5-fold upregulation in HOXB4 expression. Reconstitution efficiency of hematopoiesis in non-obese diabetic ⁄ severe combined immunodeficient mice was six-fold higher when treated with VPA than control cells. Thus apart from the use of valproic acid in neurological disorders it also has the potential to enhance hematopoiesis and therefore it can be used as an additive for *ex-vivo* expansion of $CD34^+$ cells [104].

The effect of VPA on hematopoiesis has been very well studied, but its effect on myeloid differentiation needs to be studied in detail. Different HDAC inhibitors like trichostatin A, sodium butyrate and valproic acid have shown a dose-dependent effect on myeloid differentiation [105]. Their dose must be considered while treating bone marrow disorders and other malignancies. There are reports that VPA enhances CD61/GPA population from $CD34^+$ cells when used at pharmacological concentration i.e. 100µg/ml in addition to IL-3. In the above study, HDAC inhibition by VPA significantly enhanced the generation of megakaryocytic as well as erythroid precursors from human hematopoietic precursors in the presence of IL-3 in serum-free as well as serum-containing cultures. The effect of VPA on these two

processes is mediated through IL-3 .The effect of IL-3 is more pronounced when added in combination with VPA as compared to when used alone [106-107]. HDAC inhibitors induce proliferation and differentiation by epigenetic reprogramming and this property was exploited in U937 and K562 cells [108-109]. H3 and H4 histone acetylation at gene-specific promoters was observed which differentiates them towards myeloid and erythroid lineage respectively. Its effect on megakaryocyte lineage was studied by using UT-7 Megakaryoblast leukemic cell line. It showed hyperacetylation at GPIIIa promoter which enhanced MK specific markers via ERK pathway [110]. N. Schweinfurth et al. showed similar kind of study with MEG-01 cell line in combination with ATRA. VPA alone was effective in inducing megakaryopoiesis as compared to VPA and ATRA together [111]. Recently, Toscano et al. reported that Stem cell leukemia (SCL)/ TAL-1 (T-cell acute lymphoblastic leukemia 1) is an important transcription factor which regulates MK differentiation from HSCs obtained from induced pluripotent stem cells and its over-expression increases MK-related gene expression. They carried out connectivity map analysis which revealed the same MK signatures for HDAC inhibitors as depicted by SCL/TAL1 over expression. It is the first report suggesting the direct effect of VPA on $CD34^+$ cells towards MK lineage, whereas other HDAC inhibitors like SAHA and Trichostatin A showed only an increase in CD34+ population without any effect or little effect on MK lineage [112]. It is difficult to study the the process of thrombopoiesis with primary cultures due to low number of starting MK progenitors population and availability of samples.

Overall these two pharmacological compounds, ATRA (transcription factor) and VPA (HDAC inhibitor) are potent biological agents capable of modulating gene expression to promote proliferation and differentiation of various cell types like leukemic blast, HSCs and iPSCs. They are very effective chromatin remodelling compounds and this property should be exploited to further develop superior methods for treating haematological disorders as well as for carrying out basic research

Our lab mainly focuses on *ex-vivo* expansion, differentiation and cryopreservation of HSCs, MSCs and iPSCs derived from umbilical cord blood and cord tissues [113-114].Earlier work from our lab has also shown that incorporation of two nutraceuticals i.e. Omega-3 docosahexanoic acid (DHA) and omega-6 arachidonic acid (AA) in the culture medium leads to an enhancement in the MK and platelets production from umbilical cord blood derived $CD34^+$ cells. They act as antioxidants in the medium and have an anti-apoptotic effect [115-116]. We have also used the above mentioned pharmacological compounds i.e. VPA and ATRA in our studies.VPA is being used for differentiation of MEG-01 cell line to MK and platelets which will help to decipher the cellular and molecular events involved in platelet biogenesis (manuscript under preparation).The use of VPA has also been implicated in differentiating neural stem cells. Hence, in another project we have seen its beneficial effects on differentiation of mesenchymal stem cells to neural lineage (manuscript under preparation).

Thus, VPA and ATRA have the potential to maintain stemness and also alter the stem cell fate by inducing differentiation making them effective pharmacological compounds. In the past they have been used either alone or in combination with different chemotherapeutic drugs for the treatment of haematological malignancies like AML and APL. When CD34+ cells are cultured in the presence of VPA, their proliferation is increased. VPA also enhances homing and engraftment potential of HSCs as shown by increase in CXCR4 expression in NOD/SCID

mice. They not only maintain CD34+ stem cells but also skew towards megakaryocyte lineage as shown by different cell line models like UT-7/TPO and MEG-01. All these reports underscore the role of valproic acid and ATRA in maintenance of stem cells, differentiation of primary blast cells, as well as differentiation towards MK lineage. Thus, these two compounds can be exploited in understanding the cellular and molecular mechanism underlying haematopoiesis and thrombopoiesis.

Conflict of Interest

The authors claim no conflict of interest.

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