Novel culture medium (ReproHSC™) for human hematopoietic stem cells with a small-molecule agonist of thrombopoietin receptor.

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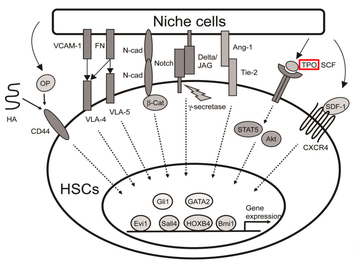
SUMMARY

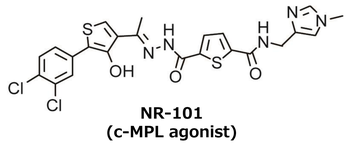
Hematopoietic stem cells (HSCs), defined by their capacity to self-renew and differentiate into all blood cell lineages, can be applied for transplantation therapy. Since a large number of HSCs are required for clinical use, improvement of techniques for expansion of HSCs ex vivo is a critical issue. Several cytokines have been used for this purpose. Thrombopoietin (TPO) is an essential cytokine that regulates megakaryocyte production and HSC proliferation via activating signaling through its receptor c-MPL. We have developed a small-molecule agonist (NR-101) of c-MPL and reported that human HSCs were expanded efficiently ex vivo with NR-101. Using a new small-molecule agonist NR-102 which is related to NR-101, we produced a novel culture medium, ReproHSC™. The cost for culture of human HSC can be reduced by using this small-molecule.

Here we demonstrated that ReproHSC™ efficiently expands human $CD34^+CD38^-$ primitive hematopoietic cells in culture and thereby enhances repopulating capacity of HSCs in NOD/SCID mice. Human blood cord $CD34^+$ cells were cultured with ReproHSC™ supplemented with only Stem Cell Factor (SCF) for 7 days. The total cell number was increased about 40-fold during culture. $CD34^+$ cells and $CD34^+CD38^-$ cells were expanded 12-fold and 8.5-fold, respectively. We then transplanted expanded cells with ReproHSC™ supplemented with SCF and flt3 ligand for 14 days into NOD/SCID mice and analyzed the SCID-repopulating $CD45^+$ cells with flow cytometry. The expanded cells established engraftment better than the fresh $CD34^+$ cells did. These results indicate that ReproHSC™ is a novel medium suitable for the expansion of HSCs ex vivo.

(1) Exp Hematol. 2009 Nov;37(11):1364-1377.e4.
Ex vivo expansion of human hematopoietic stem cells by a small-molecule agonist of c-MPL.
Nishino T, Miyaji K, Ishiwata N, Arai K, Yui M, Asai Y, Nakauchi H, Iwama A.

Fig.1. Intrinsic and extrinsic regulators of HSCs and NR-101 (a small molecule c-MPL agonist)





NR-101 (c-MPL agonist)

NR-101, a small molecule agonist of TPO receptor

Fig. 2. STAT 5 is activated to a greater extent than is STAT 3 (1).

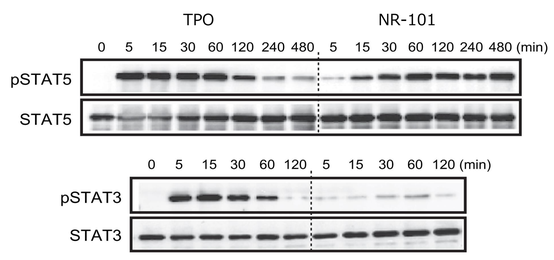
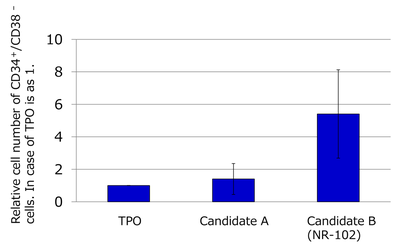
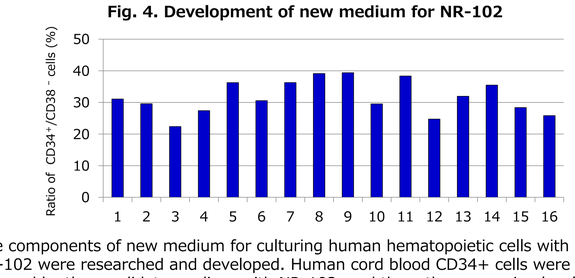


Fig.3. Development of new analogs of NR-101



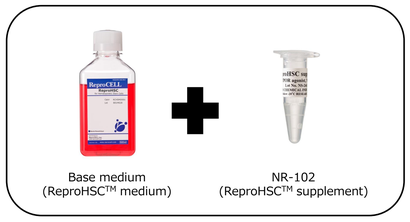
New analogs of NR-101 were identified and developed for culturing human hematopoietic cells. Human cord blood CD34+ cells were cultured in medium plus additive, and then the expression levels of CD34 and CD38 were analyzed by flow cytometry. NR-102 was determined to be the best molecule for new medium by comparison of the number of CD34+/CD38- cells.
Average ±SD (n=4).

Fig. 4. Development of new medium for NR-102



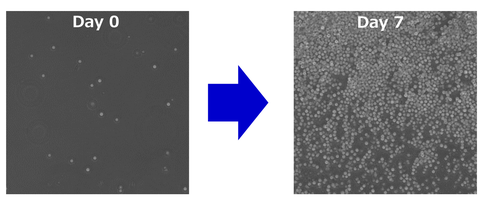
The components of new medium for culturing human hematopoietic cells with NR-102 were researched and developed. Human cord blood CD34+ cells were cultured by the candidate medium with NR-102, and then the expression level of CD34 and CD38 were analyzed by flow cytometry. We decided the optimal components of new medium from these results.

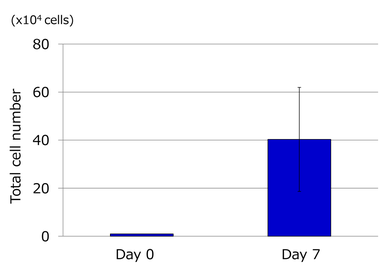
Fig. 5. ReproHSC™ , new medium for human hematopoietic stem cell



ReproHSC™ is composed of base medium (ReproHSC™ medium) and NR-102 (ReproHSC™ supplement).

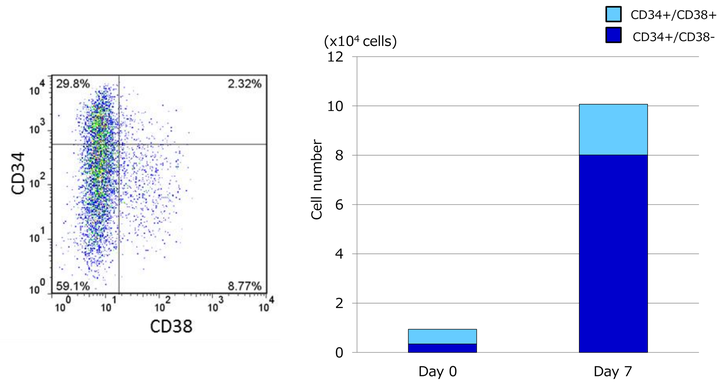
Fig. 6. The total cell number of human cord blood $CD34^+$ cells were increased by using ReproHSC™





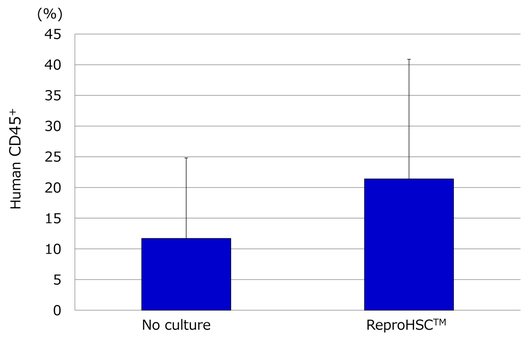
Upper : Phase contrast images, **Lower** : Total cell number
Human cord blood CD34+ cells were cultured with ReproHSC™ plus SCF (100 ng/mL) for 7 days, and then the cells were counted. The total cell number is increased about 40-fold after 7 days growth in ReproHSC™. (Average ±SD; n=14)

Fig. 7. The total cell number of CD34+/CD38- cells was increased by culturing with ReproHSC™



Left : the plot image of CD34 and CD38, **Right** : the cell number of CD34+ cells
Human cord blood CD34+ cells were cultured with ReproHSC™ plus SCF (100 ng/mL) for 7 days, and then the expression level of CD34 and CD38 was analyzed by flow cytometry. After culturing, CD34+ cells were increased about 10-fold, and 80% cells of the CD34+ cells were CD38- cells.
It is reported that HSCs are highly enriched in the CD34+/CD38- cells fraction.

Fig. 8. Transplantation into NOD/SCID mice



Human cord blood CD34+ cells were cultured in ReproHSC™ + SCF (100 ng/mL) + flk3 ligand (50 ng/mL) for 2 weeks, and the cultured cells were transplanted into NOD/SCID mice. At 8 weeks after transplantation, bone marrow cells were analyzed by flow cytometry for the presence of human CD45+cells. The number of human CD45+ cells was increased about 2-fold. These results indicate the efficiency of engraftment improved by culturing with ReproHSC™. (Average ±SD; n=5)

CONCLUSIONS

1. NR-102 was identified as a new small molecule agonist of TPO receptor (c-MPL).
2. For culture of human hematopoietic stem cells, ReproHSC™ was developed with NR-102.
3. The total cell number was increased about 40-fold after culture of human cord blood CD34+ cells in ReproHSC™ for 1 week.
4. The human cord blood CD34+ cells were increased about 10-fold after culture in ReproHSC™. In this fraction, CD38- cells were about 80%.
5. Human cord blood CD34+ cells cultured in ReproHSC™ showed higher efficiency in engraftment in NOD/SCID mice.

ACKNOWLEDGEMENT
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