Novel culture medium using a small-molecule agonist of thrombopoietin receptor.

Makoto Honda*, Taito Nishino† and Mitsuru Inamura*○
*ReproCELL Inc., Japan; †Nissan Chemical Industries, Ltd., Japan
○ Presenter

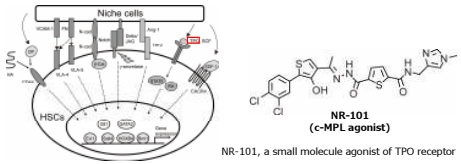
SUMMARY

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, a small molecule agonist of TPO receptor

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

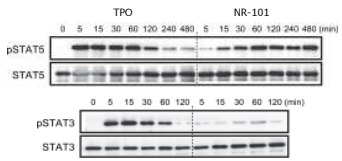
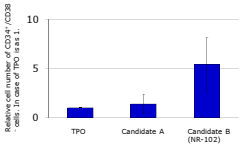
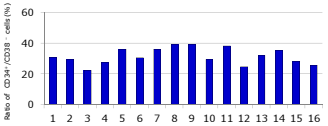


Fig.3. Development of new analogs of NR-101



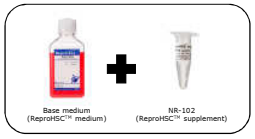
New analogs of NR-101 were identified and developed for culturing human hematopoietic cells. Human cord blood CD34+ cells were cultured in medium plus additive, and then the expression levels of CD34 and CD38 were analyzed by flow cytometry. NR-102 was determined to be the best molecule for new medium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

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



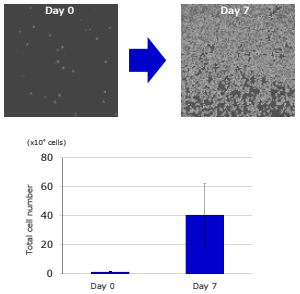
The components of new medium for culturing human hematopoietic cells with NR-102 were researched and developed. Human cord blood CD34+ cells were cultured by the candidate medium with NR-102, and then the expression level of CD34 and CD38 were analyzed by flow cytometry. We decided the optimal components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

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



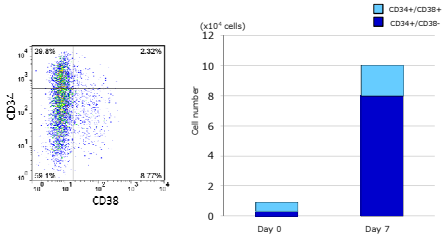
ReproHSC™ is composed of base medium (ReproHSC™ medium) and NR-102 (ReproHSC™ supplement).

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



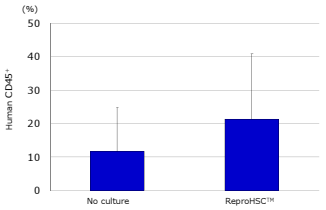
Upper : Phase contrast images, **Lower** : Total cell number
Human cord blood CD34+ cells were cultured with ReproHSC™ plus SCF (100 ng/mL) for 7 days, and then the cells were counted. The total cell number is increased about 40-fold after 7 days growth in ReproHSC™. (Average ±SD; n=14)

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
We deeply appreciate Dr. Iwama for the scientific advice and the support of some experiments in this poster. We appreciate Miss Yui for the cell preparation and the analysis of flowcytometry.