PRESS RELEASE

Embargo till Sunday 1 April 8 p.m.

TCR gene editing results in effective immunotherapy of leukaemia without the development of graft-versus-host disease (GvHD)

***Geneva, Monday April 2, 2012* – A complete genetic editing of T lymphocyte specificity was achieved by combining zinc finger nucleases - artificial molecules able to disrupt specific sequences of DNA - and lentiviral vectors - able to permanently transfer selected genes in human cells. This novel procedure allows to generate large numbers of leukaemia-specific T lymphocytes with an improved safety and efficacy profile. These results, achieved at the San Raffaele Scientific Institute in Milan, Italy, by a team led by Dr. Chiara Bonini, were published on April 1st in the *Nature Medicine* journal and received the prestigious Van Bekkum Award at the 38th Annual Meeting of the European Group for Blood and Marrow Transplantation in Geneva.**

The immune system is a very powerful system that can be exploited for the therapy of tumors. Clinical studies performed in the last decades clearly showed that selected subsets of T lymphocytes are able to recognize and eliminate tumor cells. This approach is called adoptive cellular immunotherapy and has proven effective in patients with some types of cancers, even in advanced stages.

Several T lymphocytes circulate in our body. Each T lymphocyte is specific for a particular antigen (small fragment of a protein), such as viral or fungal antigens. Thanks to their ability to respond to each antigen, T lymphocytes defend us against many diseases, eliminating, for example, viruses and fungi. Lymphocyte specificity is conferred by the "T cell receptor" (TCR), a molecule present on the surface of T lymphocytes that consists of two linked protein chains. Each T lymphocyte expresses a single TCR, different from that of other T cells present in the same individual. T lymphocytes specific for tumor antigens are able to kill tumor cells, however, they are very rare and often not enough to eradicate the tumor.

Thanks to recent advances in molecular medicine, it is now possible to redirect patients' T lymphocyte specificity towards tumor antigens by the transfer of genes encoding for a tumor-specific TCR, previously isolated from rare tumor-specific lymphocytes. This procedure, called "TCR-gene transfer" allows to generate a large number of T lymphocytes specific for a given tumor. These *genetically modified* tumor-specific lymphocytes, however, differ from the *natural* ones because they carry two different TCRs, the endogenous (already present before gene transfer) and the exogenous tumor-specific TCR which was introduced by genetic manipulation. The presence of two TCR limit the efficacy and safety of this therapy: The tumor-specific TCR, indeed, must compete with endogenous to access to the cell membrane and thus to be able to recognize the tumor. The lymphocytes generated with this technology are therefore less effective than the rare natural counterparts. Moreover, since each TCR is formed by two chains, lymphocytes engineered by TCR-gene transfer express four different chains, that can incorrectly pair and give rise to TCR with unpredictable specificity, that could possibly recognize and damage healthy tissues of the patient, causing autoimmune reactions or GvHD.

Dr. Chiara Bonini, head of the Experimental Hematology Unit and expert on T-cell gene therapy, San Raffaele, explains: "The aim of the work is to overcome these limitations by the development of "TCR-gene editing", a novel procedure, by which the endogenous TCR can be completely substituted with the tumor-specific TCR, allowing to generate a large number of lymphocytes that uniquely express high levels of the tumor-specific TCR. **This technology allows to produce, therefore, potentially for each patient, a large number of tumor-specific T lymphocytes as effective and safe as the natural anti-tumor T lymphocytes."**

This achievement was possible thanks to the collaborative effort of Dr. Chiara Bonini and Pr. Luigi Naldini, Head of San Raffaele Telethon Institute of Gene Therapy (TIGET) and leader on lentiviral vector technology and thanks to a partnership with Sangamo Biosciences, Inc., a leading Company on Zinc Finger Nucleases (ZFN). ZFN are artificial molecules able to recognize specific DNA sequences (previously selected by scientists) and able to produce breaks in DNA double helix. Upon sealing the break the genetic information is disrupted, making the cell incapable of producing the protein encoded by the gene targeted by ZFN. ZFN specific for the endogenous TCR genes were designed and

delivered to human T lymphocytes. Once the endogenous TCR was disrupted, it was then possible to transfer a gene encoding the TCR specific for a leukaemia antigen by lentiviral vectors, and obtain a large number of lymphocytes capable of recognizing and eliminating human leukemic cells, in the absence of unwanted GvHD in experimental animal models.

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The next step for this innovative strategy, still in the preclinical phase of validation, is the production of reagents and protocols suitable for clinical application.

About the European Group for Blood and Marrow Transplantation (EBMT)
The EBMT is a non-profit organisation that was established in 1974 in order to allow scientists and physicians involved in clinical bone marrow transplantation to share their experience and develop co-operative studies. The EBMT aims to promote all aspects associated with the transplantation of haematopoietic stem cells from all donor sources and donor types including basic and clinical research, education, standardisation, quality control, and accreditation for transplant procedures.
For further information about the EBMT, please visit the website: www.ebmt.org

About the Van Bekkum award
The Van Bekkum award is the most prestigious EBMT award. It is presented to the lead author of the best abstract submitted to the physician's programme and is selected by the EBMT Board. The award of 3,000€ is supported by the EBMT.

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