# ANTISENSE SEQUENCES AND ANTAGOMIR 155 IN THERAPY FOR B LYMPHOMAS OVEREXPRESSING MIR-155: PRECLINICAL MODELS AND IDENTIFICATION OF TARGET MRNAS

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MicroRNAs (miRNAs) are a conserved class of small noncoding RNAs (22-25 nucleotides), which modulate gene expression at post-transcriptional level by base pairing to the 3'UTR of the target mRNAs, thus causing messenger degradation or inhibiting its translation. Experimental evidence indicate that several miRNAs are deregulated in human tumors. MIRN155 has been shown to be highly expressed in a variety of human B cell lymphomas, especially diffuse large B cells, Hodgkin, and a subset of Burkitt lymphomas. Its expression is physiologically increased in activated B and T cells and it plays a key role in regulating the homeostasis and function of the immune system. Enforced expression of MIRN155 in haematopoietic cells affects both myeloid and erythroid differentiation. Furthermore, it has been recently demonstrated that its sustained expression in mouse haematopoietic stem/progenitor cells leads to a myeloproliferative disorder in the bone marrow and perturbs peripheral blood cell populations. Altogether these results indicate that miR-155 may play a relevant role in both lymphopoiesis and myelopoiesis.

To test this hypothesis, we first used a computational and bioinformatics-based approach to predict the putative targets involved in the control of normal haematopoiesis and haematological malignancies. Initially, we focused on the functional role of MIRN155 in megakaryopoiesis, as evaluated in unilineage megakaryocytic culture (MK) of cord blood (CB) CD34+ HPCs. Our results indicated that the decline of MIRN155 during megakaryopoiesis is an important prerequisite for MK gene expression, proliferation and differentiation. In fact, ectopic expression of MIRN155 in megakaryocytes down-regulates Ets-1 and Meis1, two transcription factors known to play a major role in megakaryopoiesis, by suppressing translation of their mRNAs. We are currently analysing the role of miR-155 in the other hematopoietic *lineages* to assess the possibility of a direct correlation between miR-155 overexpression and haematological malignancies. To this end we prepared a lentiviral vector harboring miR-155 and the GFP gene to efficiently transduce hematopoietic stem/progenitor cells. The functional effects of MIRN155 knockdown will be also analysed. Endogenous miRNA will be blocked by anti-MIRN155 transfection. Anti-miRNAs are 2'-O methyl-antisense oligonucleotides that bind and irreversibly inactivate miRNAs providing a valuable tool to specifically inhibit miRNAs function.

Recently published reports demonstrated that several miRNAs are differentially expressed during hematopoiesis and their specific expression modulate hematopoietic *lineages* differentiation indicating that miRNAs-mediated gene regulation play an important role in the developmental fate regulation of multipotent hematopoietic progenitor cells. Therefore, we are also analysing the functional role of other miRNAs to identify new set of genes and regulatory circuitries involved in the control of normal and malignant hematopoiesis.

## Publications of the project

Felli N, Pedini F, Romania P, Biffoni M, Morsilli O, Castelli G, Santoro S, Chicarella S, Sorrentino A, Peschle C, Marziali G. MicroRNA 223 dependent expression of LMO2 controls normal erythropoiesis. *Haematologica* 2008. Submitted.

Romania P, Lulli V, Pelosi E, Biffoni M, Peschle C, Marziali G. MicroRNA 155 modulates megakaryopoiesis at progenitor and precursor level by targeting Ets-1 and Meis1 transcription factors. *Br J Haematol* 2008;143(4):570-80.