IDENTIFICATION OF NEW THERAPEUTIC TARGETS THROUGH CYTOTOXICITY STUDIES OF NATURAL OR SYNTHETIC MOLECULES USING EXPERIMENTAL MODELS OF CANCER, HEMOGLOBINOPATHIES AND HIV INFECTION

1) The failure of chemotherapeutic treatments against different types of cancer is mainly due to the onset of drug resistance phenomena.

GOALS
It follows the importance of identifying new active molecules against different tumor targets in order to allow therapeutic associations and minimize the onset of resistant tumor mutants.

METHODS
The human K562 cell line of chronic myeloid leukemia is an excellent in vitro model to identify potential antiproliferative molecules to be analyzed both in reference and in association with the Imatinib molecule, currently used in antitumor therapy. Likewise, cytotoxicity studies may use tumor cell lines derived from other types of cancer, such as invasive melanoma (Colo38), cervix carcinoma (HeLa), breast carcinoma (MDA-MB231, MCF-7).

2) The reactivation of the expression of fetal hemoglobin (HbF) has been proposed as a possible therapeutic strategy of β-hemoglobinopathies. Although several HbF inducers have been tested in clinical trials, only hydroxyurea (HU) has received FDA approval.

GOALS
Although treatment with HU produced adequate HbF levels in only half of sickle cell anemia and was ineffective in patients with beta-thalassemia, the beneficial effects of this approach encourage the search for new molecules capable of inducing HbF.

METHODS
Treatment of K562 cells with natural substances derived from plants or molecules purified from natural sources to evaluate the accumulation kinetics of HbF and verify if there is a dose-response relationship. The reactivation will be compared with that of known inductors such as HU and rapamycin. Natural mixtures with proven activity will be characterized by GC-MS analysis to identify specific molecules capable of reactivating HbF expression.

3) It is essential to identify new drugs to combat drug-resistant HIV virus strains, especially molecules that can interfere with viral functions other than those targeted by antiretroviral drugs currently in use. Despite the central role played by Tat protein in the transcription of HIV, research has never been carried out on plant extracts that could hinder this important viral function.

GOALS
To evaluate the possible interference of plant extracts with the Tat/TAR-RNA interaction and with the transcription of the HIV-1 LTR induced by the recombinant Tat protein. To study the chemical composition of the active extracts by GC/MS analysis to identify molecules that once purified maintain the biological effect.

METHODS

Analysis of the interference of plant extracts with the formation of the Tat/TAR-RNA complex by Electrophoretic Mobility Shift Assay. The effect of plant extracts on the transcription of the HIV-1 LTR, induced by the recombinant Tat protein, will be analyzed using HL3T1 cells: they possess, integrated into the genome, an LTR-CAT cassette of HIV-1, transcribed at high level in the presence of Tat protein.

INSTRUMENTS AND METHODS Basic equipment for cell cultures. MTT assay, ELISA reader, analysis of Hb accumulation by means of benzidine staining, instrumentation for gel electrophoresis and for image acquisition, thermal cycler, RT-PCR real-time quantitative system to study the expression of genes involved in proliferation, differentiation, cellular invasiveness, FACS analysis for the study of cell cycle, apoptosis and cell differentiation markers.

SUBJECTS

Biochemistry, Cellular Biology, Molecular Biology and Analytical Chemistry.

WORKING GROUP

Giordana Feriotto
Carlo Mischiati (Department of Biomedical and Specialist Surgical Sciences)
Federico Tagliati (Department of Biomedical and Specialist Surgical Sciences)

COLLABORATIONS

Dr. N. Marchetti (Department of Chemical and Pharmaceutical Sciences, University of Ferrara), Prof. A. Cavazzini (Department of Chemical and Pharmaceutical Sciences, University of Ferrara), Dr. F. Casciano (LTTA Center, University of Ferrara), Prof. S. Beninati (Department of Biology, Tor Vergata University of Rome), Dr. L. Maiolo (Department of Chemistry and Chemical Technologies, University of Calabria), Dott. V. Costa (Department of Chemical and Pharmaceutical Sciences, University of Ferrara).