New Jersey State Commission on Cancer Research
LAY ABSTRACT OF RESEARCH PROJECT

NAME OF PRINCIPAL INVESTIGATOR/PROGRAM DIRECTOR: **Bernadette Cracchiolo**

Project Title: **Genital intraepithelial neoplasia and hypusination**

Description: **A novel molecular target for anticancer drug development, and its response to experimental treatment with a topical, FDA-approved inhibitor, are visualized by specific antibodies in biopsies of female and male anogenital cancers and their obligatory early stage precursors.**

It is the prevailing wisdom, even in the biomedical community, that genes encode and specify all of the building blocks ('amino acid residues') that shape the structure of proteins and in this way, determine their function in health and disease. However, numerous proteins are known to contain amino acids that are not encoded or specified by their genes. Still more surprising, in many cases these unencoded residues are the sole determinant of a protein's biological role. For example, the major proteins of the human body, the collagens, contain in every third position the amino acid hydroxyproline, which determines their structure and function; yet there is no genetic code for hydroxyproline in any collagen gene. In general, such unencoded residues are formed from encoded ones by specialized enzymes once the respective protein has been assembled; a process generically termed 'irreversible posttranslational modification'. In fact, the function of an entire physiological system, involving various other proteins, may depend entirely on the activity of just *one* of these specialized enzymes *if* it performs an irreversible posttranslational modification of strategic importance. In such a case, the single enzyme becomes a prime target for the pharmacological control of the entire physiological system. Two drugs widely used in medical therapy exemplify this principle: the oral anticoagulants and the oral antithyroids. The former suppress the strategic posttranslational modification of clotting proteins [i.e., the carboxylation of glutamate residues in factors II, VII, IX, or X], the latter inhibit the strategic posttranslational modification of the thyroid hormone-releasing protein [i.e., the iodination of tyrosine residues in thyroglobulin].

This application rests on a significant body of evidence indicating that the uncontrollable proliferation of cancer cells, a hallmark of malignancy, cannot proceed without a strategic posttranslational modification: the enzymatic formation of the unique amino acid hypusine from a genetically encoded lysine residue within eukaryotic translation initiation factor 5A (eIF5A). Point mutation of that particular lysine in eIF5A of yeast abolishes this organism's ability to proliferate. Pharmacological inhibition of hypusine formation in cultures of normal and malignant human cells invariably arrests their proliferation. In ovarian and pulmonary cancers, marked excess of eIF5A has

been noted. Obviously, it would be highly desirable **i**) to have a drug that inhibits the specialized enzyme forming the hypusine residue, thus incapacitating eIF5A; **ii**) to have a method for the routine detection of hypusine-containing, bioactive eIF5A in biopsies; and **iii**) to use this *hypusine detection method* clinically in combination with a *hypusine-suppressing drug*.

The Principal Investigator has established these two pertinent technologies in collaboration with colleagues at NIH and NJMS-UMDNJ. Specific antibodies allow the selective detection of hypusine-containing, bioactive eIF5A in cultured cells and in biopsies of human tissue, and already have been successfully employed by the Principal Investigator to analyze precancerous and cancerous lesions of the female lower genital tract (vulva and cervix), invariably revealing a marked excess of hypusine-containing eIF5A in the affected tissue. The fungicide ciclopirox, a drug approved by the Food and Drug Administration (FDA) for topical application, was found by the Principal Investigator in cultured human cells to suppress the specialized enzyme that is essential for hypusine synthesis within eIF5A. Thus, ciclopirox can be applied with the intent to knock-down bioactive eIF5A in human tissue displaying an excess of bioactive eIF5A. The FDA granted approval for such a ciclopirox pilot study to the Principal Investigator.

Applying and integrating these prior achievements, the Principal Investigator pursues a dual purpose in this application. Firstly, the immunohistological distribution of hypusine-containing eIF5A in the solid cancers of the female and male genital tract, including the anal and perianal regions, will be established and related to clinical stage and histological grade. Secondly, in the FDA-approved pilot trial, the tissue reaction and the clinical response to ciclopirox will be ascertained when topically applied to precancerous, hypusine-containing eIF5A - overexpressing lesions of the vulva. Such lesions eventually evolve into vulvar cancer, which, though rare by itself, is a human model for the many disorders and malignancies caused by infection with cell proliferation-disrupting human papillomavirus (HPV).