

**BIOGRAPHICAL SKETCH**

Provide the following information for the key personnel and other significant contributors in the order listed on Form Page 2.  
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NAME Ryszard Kole		POSITION TITLE Professor	
eRA COMMONS USER NAME			
EDUCATION/TRAINING (Begin with baccalaureate or other initial professional education, such as			
INSTITUTION AND LOCATION	DEGREE (if applicable)	YEAR(s)	FIELD OF STUDY
University of Warsaw, Poland	BS (equiv)	1964-1968	Chemistry
University of Warsaw, Poland	MS	1968-1969	Chemistry
Institute of Biochem, Biophys, Pol. Acad. of Sciences	PhD	1969-1976	Natural Sciences
Yale University, New Haven, CT, USA	Post-doc	1977-1983	Molecular Biology

**A. Positions and Honors.** List in chronological order previous positions, concluding with your present position. List any honors. Include present membership on any Federal Government public advisory committee.

**Research and Professional Experiences:**

- 1970-1977: Positions equivalent to research associate and senior research associate. Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Warsaw, Poland.
- 1977-1983: Postdoctoral positions with Dr. Sidney Altman and Dr. Sherman Weissman (associate, research staff, fellow), Yale University, Departments of Biology and Human Genetics.
- 1983-1988: Assistant Professor, Department of Pharmacology, University of North Carolina at Chapel Hill.
- 1983-present: Member, Lineberger Cancer Research Center, University of North Carolina at Chapel Hill.
- 1986-present: Member, Curriculum in Genetics, University of North Carolina at Chapel Hill.
- 1988-1996: Associate Professor, Department of Pharmacology, University of North Carolina at Chapel Hill.
- 1996-present: Professor, Department of Pharmacology; University of North Carolina at Chapel Hill.

**Honors and Awards:**

Recipient of NIH Postdoctoral Fellowship. 1980-1982.  
American Cancer Society Junior Faculty Award. Dec. 1983-Nov. 1986.  
Kenan Research leave, University of North Carolina at Chapel Hill, 1991.

**Professional Service:**

Study Sections: NIH Biochemistry (1996), Molecular Biology (1997), ad hoc reviewer.  
NIH SBIR Study Section, Member, 1999-present; American Cancer Society, 2005-present

Editorial Boards: Acta Biochemica Polonica, 1995 – present; Antisense & Nucleic Acid Drug Development, 1997 – 2003; Oligonucleotides 2004-present

**B. Selected peer-reviewed publications (in chronological order). Do not include publications submitted or in preparation.**

Dominski, Z. and Kole, R. (1993) Restoration of correct splicing in thalassemic pre-mRNA by antisense oligonucleotides. Proc. Natl. Acad. Sci. USA. 90:8673-8677.

- Dominski, Z. and R. Kole. (1994) Identification and characterization by antisense oligonucleotides of exon and intron sequences required for splicing. *Mol. Cell. Biol.* 14:7445-7454.
- Sierakowska, H., M.J. Sambade, S. Agrawal, and R. Kole. (1996) Repair of thalassemic human  $\beta$ -globin mRNA in mammalian cells by antisense oligonucleotides. *Proc. Natl. Acad. Sci. USA.* 93:12840-12844.
- Lewis, J., B. Yang, R. Kim, H. Sierakowska, R. Kole, O. Smithies, and N. Maeada. (1998) A common human  $\beta$ -globin splicing mutation modeled in mice. *Blood* 91:2152-2156.
- Gorman, L., D. Suter, V. Emerick, D. Schumperli and R. Kole. (1998) Stable Alteration of pre-mRNA splicing patterns by modified U7 snRNA. *Proc. Natl. Acad. Sci. USA.* 95:4929-4934.
- Kang, S.H., M. J. Cho, and R. Kole. (1998) Upregulation of the luciferase gene expression with antisense oligonucleotides: implications and applications in functional assay development. *Biochemistry* 37:6235-6239.
- Wilton, S.W., F. Lloyd, K. Carville, S. Fletcher, K. Honeyman, S. Agrawal and R. Kole (1999) Specific removal of nonsense mutation from the mdx dystrophin mRNA using antisense oligonucleotides. *Neuromuscular Disorders* 9:330-338.
- Sierakowska, H., M.J. Sambade and R. Kole (1999) Sensitivity of splice sites to antisense oligonucleotides. *RNA.* 5:369-377
- Schmajuk, G., H. Sierakowska and R. Kole (1999) Antisense oligonucleotides with different backbones: Modification of splicing pathways and efficacy of uptake. *J. Biol. Chem.* 274:21783-21789
- DeLong, R.K., H. Yoo, S.K. Alahari, M. Fisher, S.M. Short, S.H. Kang, R. Kole. V. Janout, S.L. Regan and R.L. Juliano (1999) Novel cationic amphophiles as delivery agents for antisense oligonucleotides. *Nucl. Acids Res.* 27:3334-3341.
- Friedman, K.J., J. Kole, J.A. Cohn, M.J. Knowles, M.J. Silverman and R. Kole (1999) Correction of aberrant splicing of CFTR gene by antisense oligonucleotides. *J. Biol. Chem.* 274:36193-36199.
- Sierakowska, H. L. Gorman, S-H. Kang and R. Kole (2000) Antisense oligonucleotides and RNAs as modulators of pre-mRNA splicing. *Meth. Enzymol.* 313:506-521.
- Lacerra, G., H. Sierakowska, C. Carestia, S. Fucharoen, J. Summerton, D. Weller and R. Kole. (2000) Restoration of hemoglobin A expression in erythroid cells from peripheral blood of thalassemic patients. *Proc. Natl. Acad. Sci.* 97: 9591-9596.
- Gorman, L., D. Mercatante and R. Kole (2000) Correction of aberrant splicing by modified U7, U6 and U1 snRNAs. *J. Biol. Chem.* 275:35914-35919
- Mercatante, D., C. Bortner, J. Cidowski and R. Kole. (2001) Modification of Alternative Splicing of Bcl-x Pre-mRNA in Prostate and Breast Cancer Cells: Analysis of Apoptosis and Cell Death. *J. Biol. Chem.* 276: 16411-16417.
- Kole, R. and P. Sazani . (2001) The antisense effects in the cell nucleus. Modification of splicing. *Curr. Opin. Mol. Ther.* 3:229-234
- Sazani, P., S-H. Kang, M.A., Maier, C. Wei, J. Dillman, J. Summerton, M. Manoharan and R. Kole (2001) Nuclear antisense effects of neutral, anionic and cationic oligonucleotides. *Nucl. Acids Res.* 29:3965-3974.
- Tian, H., and R. Kole (2001) Strong RNA splicing enhancers identified by a modified method of cycled selection interact with SR protein. *J. Biol. Chem.* 276:33833-33839.
- Kole, R., and D. Mercatante (2001) Pre-mRNA splicing as a target for antisense oligonucleotides. In: *Antisense drug technology: principles, strategies and applications*, Crook, S.T. ed., Marcel Dekker, N.Y.N.Y. pp. 517-540.
- Gemignani, F., S. Landi, D.M. DeMarini, and R. Kole (2001) Spontaneous and MNNG-Induced Reversion of an EGFP Construct in HeLa cells: an assay for observing mutations in living cells by fluorescent microscopy. *Hum. Mut.* 18:526-34
- Suwanmanee, T., H. Sierakowska, G. Lacerra, S. Kirby, C. Walsh, S. Fucharoen, and R. Kole (2002) Restoration of Human  $\beta$ -Globin gene expression in murine and human IVS2-654 thalassemic erythroid cells by free uptake of antisense oligonucleotides. *Mol. Pharm.* 62:545-53.
- Suwanmanee, T., H. Sierakowska, S. Fucharoen, and R. Kole. (2002) Repair of a splicing defect in erythroid cells from patients with  $\beta$ -thalassemia/HbE disorder. *Mol. Ther.* 6:718-726.
- Mercatante, D.R., Mohler JL and R. Kole. (2002) Cellular Response to an Antisense-mediated Shift of Bcl-x Pre-mRNA Splicing and Antineoplastic Agents. *J Biol Chem.* 277:49374-49382.

- Sazani, P., F. Gemignani, S-H Kang, M. M. Maier, M. Manoharan, M. Persmark, D. Bortner and Ryszard Kole (2002). Functional analysis of modified oligonucleotides in vivo. Systemic delivery, biodistribution and antisense effects. *Nature Biotech.* 20:1228-1233
- Gemignani, F., P. Morcos and R. Kole (2002) Temperature dependent alternative splicing of thalassemic b-globin pre-mRNA in vivo. *Nucl. Acids. Res.* 30: 4592-4598
- Sazani, P., and R. Kole (2003) Modulation of alternative splicing by antisense oligonucleotides. *Prog Mol Subcell Biol.* 31:217-239.
- Vacek, M., H. Ma, F. Gemignani, G. Lacerra, T. Kafri and R. Kole. (2003) High-level expression of hemoglobin A in human thalassemic erythroid progenitor cells following lentiviral vector delivery of an antisense snRNA. *Blood* 101:104-11.
- Sazani P, Astriab-Fischer A, Kole R. (2003) Effects of Base Modifications on Antisense Properties of 2'-O-Methoxyethyl and PNA Oligonucleotides. *Antisense Nucleic Acid Drug Dev.* 13:119-28.
- Vacek M, Sazani P, Kole R. (2003) Antisense-mediated redirection of mRNA splicing. *Cell Mol Life Sci.* 60:825-33.
- Bristow CL, Mercatante DR, Kole R. (2003) HIV-1 preferentially binds receptors co-patched with cell surface elastase. *Blood.* 102:4479-86
- Sazani P, Kole R. (2003) Therapeutic potential of antisense oligonucleotides as modulators of alternative splicing. *J Clin. Invest.* 112:481-6.
- Sazani P, Astriab-Fischer A, Kole R. (2003) Effects of base modifications on antisense properties of 2'-O-methoxyethyl and PNA oligonucleotides. *Antisense Nucleic Acid Drug Dev.* 13:119-128.
- Kole, R., Vacek, M., Williams, T (2004) Modification of alternative splicing by antisense therapeutics. *Oligonucleotides.* 14:65-74.
- Williams, T and Kole, R. Analysis of prostate specific membrane antigen splice variants in LNCap cells. *Oligonucleotides In press.*

**C. Research Support.** List selected ongoing or completed (during the last three years) research projects (federal and non-federal support). Begin with the projects that are most relevant to the research proposed in this application. Briefly indicate the overall goals of the projects and responsibilities of principal investigator identified above. Research Projects Ongoing or Completed During the Last 3 Years

#### ONGOING

- NIH Program Project (PA-95) "A novel strategy for evaluating the in vivo effectiveness of antisense oligonucleotides and genes." April 1, 2005-March 31, 2010. Project Director. Annual direct costs: \$195,000. Development of animal models for investigation of antisense oligonucleotides
- NIH STTR Grant, Phase I Fast Track. "Antisense Treatment for Thalassemia. Preclinical Study. October 1, 2004- August 30, 2005. Principal Investigator. Total annual costs 240,095 Investigation of splice switching oligonucleotides as potential treatment for thalassemia. In vivo experiments in a mouse model of a thalassemic splicing defect.
- NIH STTR Grant, Phase II Fast Track. "Antisense Treatment for Thalassemia. Preclinical Study. September 1, 2005- September 30, 2007. Principal Investigator. Total annual costs 577,859 Investigation of splice switching oligonucleotides as potential treatment for thalassemia. In vivo experiments in a mouse model of a thalassemic splicing defect.
- Cooley's Anemia Foundation "Restoration of correct splicing in thalassemic pre-mRNA by antisense oligonucleotides." July 1, 2004 - June 30, 2006. Total award: \$60,000 (Postdoctoral fellowship for Dr. Thipparat Suwanmanee)

#### COMPLETED

- Ercole Biotech, Inc. SRA. "Modulation of splicing by antisense oligonucleotides" June 1, 2003- May 31, 2004. Principal Investigator. Annual total costs; \$290,000. Investigation of various oligonucleotides in cell culture and mouse models.
- NIH 2-R01-HL51940-07 (Kole) "Restoration of Globin Gene Expression in Thalassemia" 12/01/98 – 2/28/02. Investigation of splice switching oligonucleotides as potential treatment for thalassemia. Cell culture experiments mouse model of a thalassemic splicing defect and in thalassemic patient cells.