BIOGRAPHICAL SKETCH

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NAME: Luca Cartegni

eRA COMMONS USER NAME (credential, e.g., agency login): CARTEGNL

POSITION TITLE: Associate Professor

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
Universita' degli Studi di Pavia, Italy	Laurea (MS equivalent	1991	Biological Sciences
Universita' degli Studi di Pavia, Italy	Ph.D.	1996	Molecular Biology
Istituto di Genetica Molecolare, CNR, Pavia, Italy	Post-doc	1996-1997	Molecular Biology
Cold Spring Harbor Laboratory, NY	Post-doc	1997-2004	Molecular Biology

A. Personal Statement

My past and current studies have been aimed at identifying **the molecular and cellular mechanisms of posttranscriptional regulation in disease** (particularly RNA splicing and polyadenylation), and at **developing new strategies and drugs to exploit RNA biology to treat genetic diseases and cancer**, especially in the context of drug resistance.

My lab has been addressing this questions from three different directions: 1) identification of the aberrant RNA processing events; 2) characterization of the mechanisms involved, including cis-element and trans-factors responsible; 3) development of technologies to correct (or exploit) RNA processing events for therapeutic purposes.

More specifically, I have developed methodologies to identify and/or predict regulatory exonic splicing enhancers and, more recently I have used them to map and characterize functional binding sites for RNA–binding proteins that play a role in pathological aberrant splicing events in genetic diseases or in cancer, such as for example hnRNPH protein in Glioblastoma Multiforme. Further, I have developed antisense-based compounds to control the expression of such proteins in vitro and to specifically modulate the splicing patterns of some of their targets.

In addition, we applied this same splicing redirection approach *in vitro* and *in vivo* to induce expression of antagonistic variant of pathological proteins such as the oncogene STAT3, while a parallel approach was developed to specifically activate intronic polyadenylation sites to express soluble decoy Receptor Tyrosine Kinases (sdRTKs) instead of their full-length counterparts. These sdRTK can act as dominant-negative to block the signaling of the full length RTKs (like VEGFR, EGFR, HER2, MET), with broad applications as investigational tools or potential cancer therapeutics, especially in the context of tumors which have acquired resistance to more standard approaches, like small molecule Tyrosine Kinase Inhibitors.

My overall research goal is to better understand the general role of aberrant RNA processing in cancer and to harness this understanding to develop a platform of antisense-based molecular tools, to be used both as investigational implements and as powerful therapeutics in cancers that do not respond to conventional treatments.

I have authored or co-authored 35 peer-review papers, several of which are well cited and published in highimpact journals such as Nature Genetics, Nature Structural Biology, Nature Biotechnology, Nature Review Genetics, Molecular Cell, PNAS, EMBO Journal, America Journal of Human Genetics and others. My papers have accumulated over 6,000 citations in peer-reviewed journals, for a H-index of 24 and an i10-index of 30 (Google Scholar). My research has been supported by grants from NIH, DOD and other sources.

a. **Cartegni L**, Krainer AR. Disruption of an SF2/ASF-dependent exonic splicing enhancer in SMN2 causes spinal muscular atrophy in the absence of SMN1. *Nature Genet*. 30(4): 377-84 (2002).
b. **Cartegni L**, Krainer AR. Correction of disease-associated exon skipping by synthetic exon-specific activators. *Nature Struct. Biol*., 10(2): 120-125 (2003).
c. Zammarchi F, de Stanchina E, Supakorndej T, Martires K, Riedel E, Corben A, Bromberg J and **Cartegni** L. Anti-tumorigenic potential of STAT3 alternative splicing modulation. *Proc Natl Acad Sci USA* 108 (43) 17779-17784 (2011) PMC3203802
d. Vorlova S, Rocco G, LeFave C, Jodelka F, Hastings M, Henke E, **Cartegni L**. Induction of antagonistic soluble receptor tyrosine kinases by intronic PolyA activation. *Mol Cell*.; 43(6):927-39 (2011)

Ongoing and recently completed projects that I would like to highlight include:

7RO1CA233897
Cartegni (PI)
12/1/18-11/30/24
Targeting refractory EGFR-Driven Tumors by induction of dominant-negative EGFR splicing Variants

7RO1CA219689
Cartegni (PI)
12/8/18 – 11/30/23
Therapeutic potential of antitumorigenic soluble MET variants induced by splicing interference

B. Positions, Scientific Appointments, and Honors

Positions and Scientific Appointments

2023	Codify, Consultant
2022	Sanavia, Consultant
2019-present	Stoke Therapeutics, Scientific Advisory Board
2018	New Jersey Health Foundation Excellence in Research Award
2016-present	Member, Cancer Institute of New Jersey
2014-present	Graduate Faculty of Cellular and Molecular Pharmacology, Rutgers University
2014-present	Graduate Faculty of Microbiology and Molecular Genetics, Rutgers University
2013-present	Associate Professor, Chemical Biology, School of Pharmacy, Rutgers University, NJ
2007-2013	BTC member, Brain Tumor Center, MSKCC
2005-2013	Assistant Professor, Gerstner Sloan-Kettering School, MSKCC
2005-2013	ETC member, Experimental Therapeutic Center, MSKCC
2004-2013	Bressler Scholar (Alfred W. Bressler Scholars Endowment Fund recipient)
2004-2013	Assistant Professor, Weill Graduate School of Medical Sciences
2004-2013	Assistant Member, Molecular Pharmacology, and Chemistry Program, Memorial Sloan-Kettering Cancer Center (MSKCC), NY
2003	Instructor, ICGEB (International Center for Genetic Engineering and Biotechnology), Ph.D. Program Lecture Series, Trieste (Italy)
2001-2003	Senior Fellow, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
2000-2001	Post-Doctoral Fellow, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
2000	Adjunct Assistant Professor, Queens College of CUNY Graduate School Program, Queens, NY
1997-2000	HFSP Fellow, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
1997	LIBA Fellow, Cold spring Harbor Laboratory, Cold Spring Harbor, NY
1996-1997	Telethon Foundation Fellow, Istituto di Genetica Molecolare, CNR, Pavia, Italy (then called IGBE)
1991	Anna Villa Rusconi Foundation Fellowship

C. Contribution to Science

1. **Characterization of exonic splicing enhancers and other splicing elements and predominant role of splicing mutations in diseases.** Alternative splice site selection is combinatorially controlled by the spliceosome, which recognizes the splice sites themselves, and by additional trans-acting splicing factor (RNA binding proteins) that bind to a number of exonic and intronic cis-elements on the pre-mRNA. As a postdoctoral fellow in Adrian Krainer laboratory in Cold Spring Harbor, I iworked on the identification of exonic splicing enhancers (ESE) recognized by a subset of SR protein splicing factors, and developed a matrix-based algorithm to predict their occurrence in pre-mRNA. I then transformed this into a widely used, public web resource for the identification of ESE in pre-mRNAs (ESEfinder). Given the prevalence and importance of ESEs, a strong prediction was that many mutations in disease genes and cancer, formerly identified as silent, missense or nonsense, were in fact primarily splicing mutations. This prediction, discussed in a highly-cited Nature Reviews Genetics paper, proved correct and has contributed to significantly alter the interpretation of mutations in genetic diseases and cancer. I since authored and co-authored several papers that explore this concept. Most recently, in collaboration with Raffaella Sordella at CSHL, we identified mutations in tumor suppressor p53 that affect its splicing, and generate gain-of function p53 variants (PSI variants) rather than loss-of-function effect.

a. Liu HX, **Cartegni L**, Zhang MQ, Krainer AR. A mechanism for exon skipping caused by nonsense or missense mutations in BRCA1 and other genes. Nature Genet. 27(1):55-8 (2001).
b. **Cartegni L**, Chew SL, Krainer AR. Listening to silence and understanding nonsense: exonic mutations that affect splicing. *Nature Rev. Genet*. 3(4):285-98 (2002).
c. **Cartegni L**, Wang J, Zhu Z, Zhang MQ, Krainer AR. ESEfinder: a Web resource to indentify exonic splicing enhancers. *Nucleic Acids Res*., 31(13): 3568-71 (2003). PMC169022
d. Senturk S, Yao Z, Camiolo M, Stiles B, Rathod T, Walsh AM, Nemajerova A, Krainer A, Moll UM, Lowe SW, **Cartegni** L, Sordella R p53Ψ is a transcriptionally inactive p53 isoform able to reprogram cells toward a metastatic-like state. *Proc Natl Acad Sci USA.* Aug 12;111(32):E3287-96. (2014)

2. Characterization of hnRNPs. Some of the RNA-binding proteins involved in RNA processing are part of the heterogeneous nuclear RNP family (hnRNPs), which in general behave as splicing inhibitors. As a graduate student in Pavia I worked on the biological and biochemical characterization of the prototypical hnRNPA1. More recently, in my (previous) lab at MSKCC, we identified hnRNPH1, another hnRNP, as a driver of an oncogenic switch in Glioblastoma Multiforme, and expanded its involvement in brain biology by its control of Opioid Receptor alternative splicing (in collaboration with Dr. YX Pan).

a. **Cartegni L**, Maconi M, Morandi E, Cobianchi F, Riva S, Biamonti G. HnRNP A1 selectively interacts through its Gly-rich domain with different RNA-binding proteins. *J. Mol. Biol*. 259(3):337-48 (1996)
b. Weighardt F, Cobianchi F, **Cartegni L**, Chiodi I, Villa A, Riva S, Biamonti G. A novel hnRNP protein (HAP/SAF-B) enters a subset of hnRNP complexes and relocates in nuclear granules in response to heat shock. *J. Cell Sci*. 112(10):1465-76 (1999).
c. LeFave C, Squatrito M, Vorlova S, Rocco G, Brennan C, Holland E, Pan Y, **Cartegni L**. Splicing factor hnRNPH drives an oncogenic switch in gliomas. *EMBO J*; 30(19):4084-97 (2011) PMC3209773
d. Xu J, Lu Z, Xu M, Pan L, Deng Y, Xie X, Liu H, Ding S, Hurd YL, Pasternak GW, Klein RJ, **Cartegni** L, Zhou W, Pan YX. A heroin addiction severity-associated intronic single nucleotide polymorphism modulates alternative pre-mRNA splicing of the μ opioid receptor gene OPRM1 via hnRNPH interactions. J Neurosci. 2014 Aug 13;34(33):11048-66. -13.2014. (2014)

3. **Molecular mechanisms underlying Spinal Muscular Atrophy.** Spinal Muscular Atrophy is a severe neurodegenerative disease caused by loss of expression of the SMN1 gene. An almost identical copy of SMN1, SMN2, contains only a handful of nucleotide changes, none of which affects the coding potential. However, a silent substitution in exon 7 causes a splicing defect in SMN2, which leads to a non-functional protein. I demonstrated that the splicing defect is mainly due to the disruption of an ESE recognized by splicing factor SF2/ASF (nor called SRSF1), and in a subsequent paper I showed that correction of the splicing pattern by antisense compounds could re-instate correct SMN expression. These works pioneered the possibility of treating SMA (and many other genetic

diseases) using splicing-redirection antisense compounds. A third generation iteration of this approach, developed by the Krainer lab in collaboration with Ionis Pharmaceuticals has been recently approved by the FDA, providing the first ever therapy fro SMA, and the first truly effective antisense drug on the market (however, I have not been involved with that project after I left CSHL).

a. **Cartegni L**, Krainer AR. Disruption of an SF2/ASF-dependent exonic splicing enhancer in SMN2 causes spinal muscular atrophy in the absence of SMN1. *Nature Genet*. 30(4): 377-84 (2002).
b. **Cartegni L**, Krainer AR. Correction of disease-associated exon skipping by synthetic exon-specific activators. *Nature Struct. Biol*., 10(2): 120-125 (2003).
c. **Cartegni** L, Hastings ML, Calarco JA, de Stanchina E, Krainer AR. Determinants of exon 7 splicing in the Spinal muscular atrophy genes, SMN1 and SMN2. *Am J Hum Genet*. Jan;78(1):63-77. (2006)
d. **Cartegni L**, Krainer AR. Chimeric Molecules to Modulate Gene Expression. (2000) WO/2002/038738 and PCT/US2001/047523.

4. Role of alternative splicing in cancer and development of antisense compounds to induce endogenous anti-tumorigenic variants of oncogenes. Aberrant alternative splicing plays an important and increasingly recognized role in cancer, both as a co-driver when splicing factors involved in oncogenic splicing shifts are overexpressed in cancer, as we showed to be the case for hnRNPH, or as a mechanism to generate more aggressive or drug-resistant isoforms, like in the case of Ron, MADD, ERG and many others. However, the presence of functionally antagonistic splicing variants in oncogenes can also be exploited for the development of novel therapeutic approaches. Harnessing the strategy I had developed for SMA, I adapted it to one the defining aspects of my recent research: the development of antisense compounds to reprogram oncogenes to express antagonist, dominant-negative variants instead of the pathological proteins. This was first demonstrated with STAT3, when we were able to show for the first time that the manipulation of one single splicing event in tumors could lead to full regression in vivo 9in mouse models). Receptor Tyrosine kinases also express antagonistic splicing variants. In a well-received Molecular Cell paper, we described the discovery that in fact most RTKs express soluble decoy variants (sdRTKs), encompassing only portions of the extracellular domain, and retaining ligand binding and dimerization capabilities. Thus they potentially act as dominant negative variants. Indeed, sdVEGFR variants behave as potent inhibitors of VEGF activity and block angiogenesis in vitro and in vivo. The possibility of specifically and effectively inducing these variants in vivo using antisense–based compounds has broad potential implication in RTK-targeted therapies in cancer. The studies proposed in this grant application directly follow the work described in the Molecular Cell paper.

a. Zammarchi F, de Stanchina E, Supakorndej T, Martires K, Riedel E, Corben A, Bromberg J and **Cartegni** L. Anti-tumorigenic potential of STAT3 alternative splicing modulation. *Proc Natl Acad Sci USA* 108 (43) 17779-17784 (2011) PMC3203802
b. LeFave C, Squatrito M, Vorlova S, Rocco G, Brennan C, Holland E, Pan Y, **Cartegni** L. Splicing factor hnRNP H drives an oncogenic switch in gliomas. *EMBO J*; 30(19):4084-97 (2011) PMC3209773
c. Zammarchi F, Boutsalis G, **Cartegni** L (2013) 5' UTR Control of Native ERG and of Tmprss2:ERG Variants Activity in Prostate Cancer. *PLoS One* 8(3): e49721. doi:10.1371/journal.pone.0049721 (2013)
d. Vorlova S, Rocco G, LeFave C, Jodelka F, Hastings M, Henke E, **Cartegni L**. Induction of antagonistic soluble receptor tyrosine kinases by intronic PolyA activation. *Mol Cell*.; 43(6):927-39 (2011)

5. U1-snRNP-dependent inhibition of intronic polyadenylation and other antisense approaches to target RNA. We discovered that the activation of intronic polyadenylation which we observed for RTK genes is a general phenomenon controlled by a novel U1 snRNP-dependent mechanism (this was at the same time independently discovered by the Dreyfuss lab, at U Penn). U1 snRNP is a core component of the spliceosome and normally binds to the 5' splice site, promoting splicing. However, our data show that from that position U1snRP also actively suppresses downstream intronic polyadenylation, acting as an RNA surveillance mechanism parallel to NMD, which ensures the integrity of full-length transcripts. The interference with this mechanism by antisense oligonucleotides that compete with U1 snRNP for binding to specific 5'ss upstream of actionable IPA sites is the basis for the activation of sdRTKs described in the section above, and can be applied to the induction of truncated

variants of any number of genes. In parallel to studies of the U1-dependent mechanism and development of the related antisense technology, we also started developing peptide-based delivery systems for antisense compounds, and invented a novel knock-down approach, which we called Forced Splice-Dependent NMD (FSD-NMD), that relies on the synthetic induction of out-of-frame splicing events to knock down gene expression in vitro and in vivo.

a. Vorlova S, Rocco G, LeFave C, Jodelka F, Hess K, Hastings ML, Henke E, **Cartegni L**. Induction of antagonistic soluble receptor tyrosine kinases by intronic PolyA activation. *Mol Cell*.; 43(6):927-39 (2011) *NIHMS318430*
b. L. Spraggon and Luca **Cartegni**, U1 snRNP-Dependent Suppression of Polyadenylation: Physiological Role and Therapeutic Opportunities in Cancer, *International Journal of Cell Biology*, vol. 2013, Article ID 846510, 2013. doi:10.1155/2013/846510
c. Henke E, Perk J, Vider J, de Candia P, Chin Y, Solit DB, Ponomarev V, **Cartegni L**, Manova K, Rosen N, Benezra R. Peptide-conjugated antisense oligonucleotides for targeted inhibition of a transcriptional regulator in vivo. *Nat Biotechnol*. 2008 Jan;26(1):91-100.
d. Spraggon L. and **Cartegni** L; Antisense Modulation of RNA Processing as a Therapeutic Approach in Cancer Therapy, *Drug Discovery Today. Therapeutic Strategies*, (2013), http://dx.doi.org/10.1016/j.ddstr.2013.06.002

Inventions & Patents

1. Raffaella Sordella, Luca Cartegni, Serif Senturk, 2015, Methods And Compositions For Inhibiting Growth And Epithelial To Mesenchymal Transition (Emt) In Cancer Cells; World Intellectual Property Organization Publication Number WO2015_200725
2. Cartegni L, Krainer AR. Chimeric Molecules to Modulate Gene Expression. (2000) WO/2002/038738 and PCT/US2001/047523.
3. Biamonti G, Bassi MT, Cartegni L and Riva S. Expression Vector for Mammalian Cells. (1995) Patent MI95A001281

Bioinformatics

1. Cartegni L, Zhu Z, Zhang MQ, Krainer AR. ESEfinder: splicing enhancer analysis algorythm and webtool. (http://rulai.cshl.edu/tools/ESE2/)

Complete List of Published Work available in *MyBibliography* :
http://www.ncbi.nlm.nih.gov/sites/myncbi/luca.cartegni.1/bibliography/44200043/public/?sort=date&direction=ascending